SCIENCE

VOL. 74 FRIDAY, AUGUST 7, 1931 No. 1910

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

New York City: Grand Central Terminal

Lancaster, Pa. Garrison, N. Y.

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary, in the Smithsonian Institution Building, Washington, D. C.

OBSERVATIONS UPON THE FILTERABILITY OF BACTERIA, INCLUDING A FILTERABLE ORGANISM OBTAINED FROM CASES OF INFLUENZA[1]

STUDIES IN BACTERIAL METABOLISM, CI

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THERE is a heterogenous group of formidable diseases of man and animals, including some of the most rapidly spreading infections, whose inciting agents have thus far eluded cultivation upon artificial mediums. There can be little doubt that the incitants of this group are living entities and their cultivation outside the body should, judging from past experience in bacteriology and preventive medicine, be helpful, not only in solving important problems of their life history, but also in approaching the solution of prophylactic and curative measures. A few of these "viruses" are said to have been kept alive for periods of time in presence of large amounts of blood, or pieces of tissue from recently killed animals, but by common consent, this limited, restricted development is not regarded as equivalent to cultivation in the usual bacteriological sense.

[1] The James A. Patten Lecture in Bacteriology, Northwestern University Medical School, Chicago, Illinois, July 22, 1931.

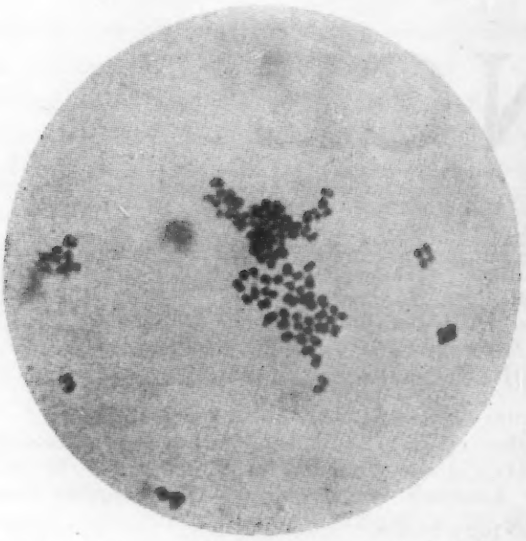

FIG. I. Diplococcus from a case of influenza, showing variation in size and intensity of staining.

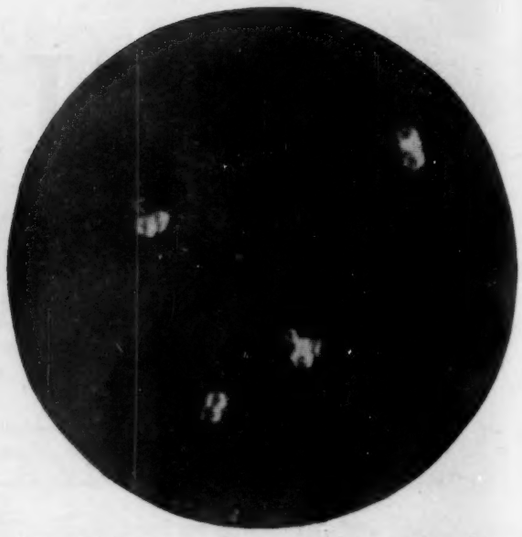

FIG. II. Diplococcus from a culture of influenza, 48 hour culture in K medium. Dark field illumination, showing granules.

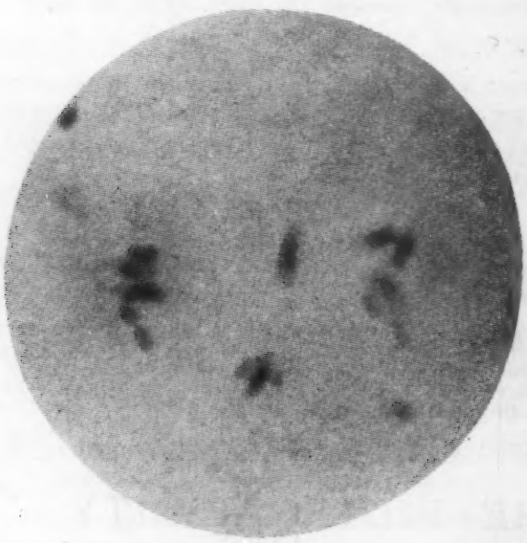

FIG. III. B. typhosus, 24 hours' growth in K medium, showing granules, and faintly staining residuum of organism.

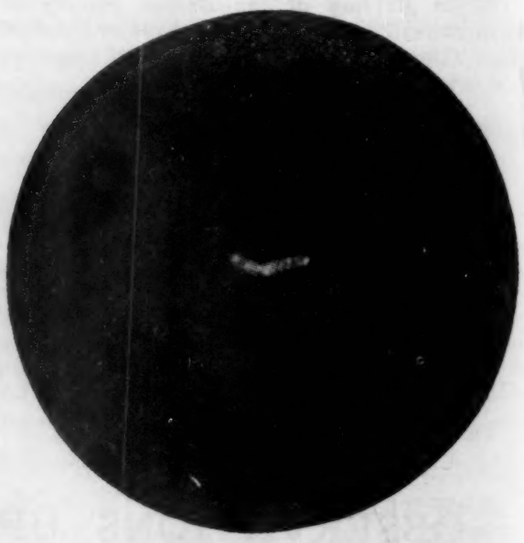

FIG. IV. B. typhosus, 48 hour culture in K medium. Dark field illumination, showing granules.

One can not but be impressed, in attempting to analyze the uniform failure to induce growth of these "viruses" in artificial mediums, with the fact that artificial mediums at present available for their cultivation depart radically from the physical nature and chemical composition of their natural habitats. Also, and this is significant, the more closely mediums approximating the nature and composition of living tissues are prepared, the more nearly has there been indication of at least some multiplication of these "viruses" outside the animal body. Comparing the artificial medium with the natural environment of these "viruses," one is struck by two outstanding differences, omitting for the present the question of inorganic constituents. The artificial medium contains protein degradation products, peptones and meat extractives, and little or no unaltered, or nearly unaltered, protein. The tissues of the body, on the contrary, contain unaltered protein, and little or no peptone or other protein degradation products, and there is clearly a difference as well as a distinction between limited life in fresh tissue or blood, on the one hand, and on the other hand true passage with almost limitless capacity for multiplication through a series of artificial cultivations. By comparison, current knowledge of the nature of cultivable microbes, their morphology, physiology and chemistry, contrasts strikingly with the paucity of information concerning the so-called "virus" diseases, of which but little is known, except objectively. And yet in this virus group are smallpox, vaccinia, measles, rabies, that extraordinarily contagious group com-

prising influenza, common cold and its clinical variants, poliomyelitis, encephalitis and several others.

A theoretical method of approach to the problem of cultivation of these refractory "viruses," therefore, would appear to be the preparation of a sterilizable medium containing unaltered, or nearly unaltered, protein, and without peptone or significant amounts of other nitrogenous constituents, to confine discussion for the present to these most significant substances.

Such a medium has been prepared.[2] In brief, the essential ingredient is small intestine of man, swine, dog or rabbit, whichever is available, thoroughly extracted with alcohol to remove water and alcohol soluble extractives, followed by extraction with benzol to remove excess of lipoidal substances. This residue, dried, can be kept indefinitely. The addition of Tyrode solution, or even normal saline to this powdered extracted substance, makes a rather turbid medium which can be autoclaved without apparent harm. The autoclaved medium (referred to hereafter for brevity as the K medium) possesses most unusual qualities. These may best be expressed by citation of a series of experiments with it.

Fresh, aseptically drawn blood from seven cases diagnosed as mild influenza, four from cases in Passavant Hospital, three from cases in Evanston Hospital, was added to K medium, previously heated, then rapidly cooled to expel air, in the proportions of 10 cc of blood to 90 cc of medium. Incubation was practiced for several days at 30 degrees C. Three cultures showed increasing cloudiness with the progress of incubation; four did not. From the three cloudy cultures, an organism was isolated. The four remaining bloods continued sterile for nine weeks. Then they were discarded. Attention is drawn in passing to the difficulty in establishing a definite clinical diagnosis of influenza in these milder, sporadic cases, which occur in inter-epidemic periods. It is not without significance, therefore, that the three positive cases discussed here were found in two entirely separate institutions, and the diagnoses were made absolutely independently by physicians living in Chicago and Evanston, respectively.

Inasmuch as there has been, very properly, much just scepticism about the cultivation of filterable organisms in general, the details, including dates, of the two positive cultures from the Passavant cases are reported here at considerable length.

Isolation of Filterable Organisms from Cases of Influenza

Eight rabbits were put in stock under observation January 2, 1931. No spontaneous illness developed during the course of the experiments that followed.

[2] The details of preparation will appear in another communication.

A. February 3, 1931. 10 cc of blood from cases No. 3 and No. 4 (Passavant), respectively, were added to 90 cc of K medium.[3] Incubation was practiced for 10 days at 30 degrees, during which time distinct cloudiness developed. (Cultures No. 3A and No. 4A.)

B. February 13, 1931. Seven loopfuls of culture from No. 3A and No. 4A, respectively, were added to 10 cc of the K medium. They were incubated at 30 degrees for 10 days. The mediums became progressively clouded. (Cultures No. 3B and No. 4B.)

C. February 23, 1931. Seven loopfuls of culture from No. 3B and No. 4B, respectively, were added to 10 cc of the K medium. Incubated at 30 degrees for 10 days. The mediums again became clouded. (Cultures No. 3C and No. 4C.)

D. March 5, 1931. Seven loopfuls of culture from No. 3C and No. 4C, respectively, were added to 10 cc of the K medium. Incubated 5 days at 30 degrees. Considerable cloudiness developed. (Cultures No. 3D and No. 4D.)

E. March 10, 1931. One half cc of cultures No. 3D and No. 4D, respectively, was injected into the ear vein of rabbits No. 3 and No. 4. At the same time, as control, 1 cc of the medium from each of three negative flasks of K medium (from cases No. 1, No. 2 and No. 5), was injected into each of three rabbits. These control rabbits remained normal.

Ea. March 11, 1931. Rabbits No. 3 and No. 4 sneezed paroxysmally, and frequently during this day and for the next four following days, with apparently unabated violence. Rectal temperatures for March 11 (24 hours after inoculation) and for March 18 (one week later), were as follows:

	Temperature March 11	Temperature March 18
No. 1 (control rabbit)	102.2	102.0
No. 2 (control rabbit)	102.1	102.0
No. 3 ("flu" rabbit)	103.4	102.8
No. 4 ("flu" rabbit)	104.0	102.5
No. 5 (control rabbit)	102.4	102.2

By the sixth day, the acuteness of the sneezing had abated. Rabbit No. 4 made an uneventful but rather slow recovery; rabbit No. 3 still sneezes paroxysmally (June 23) and appears to be slowly losing ground.

F. March 12, 1931. 12 cc of blood was drawn from rabbits No. 3 and No. 4, respectively; it was distributed as follows:

1 cc to 10 cc of K medium	=cultures No. 3Fa and No. 4Fa.
1 cc to 10 cc of dextrose broth	=cultures No. 3Fb and No. 4Fb.
10 cc to ice box for 24 hours	=blood, No. 3Fc and No. 4Fc.

Cultures No. 3Fa and No. 4Fa, after 10 days' growth at 30 degrees were plated on agar containing both dried intestine and proteose peptone.[4] Three days' anaerobic incubation followed by four days' aerobic incubation at

[3] K medium heated, and cooled rapidly each time to remove air.

[4] Proteose peptone, extracted thoroughly with absolute ethyl alcohol; meat infusion peptone agar is equally satisfactory, as was found out later.

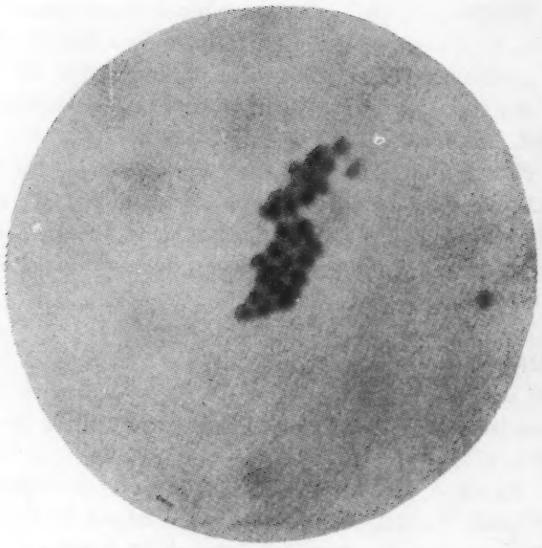

FIG. V. Staphylococcus from Staphylococcus bacteriophage. First culture on agar after K medium. Irregularities in size and staining are noticeable.

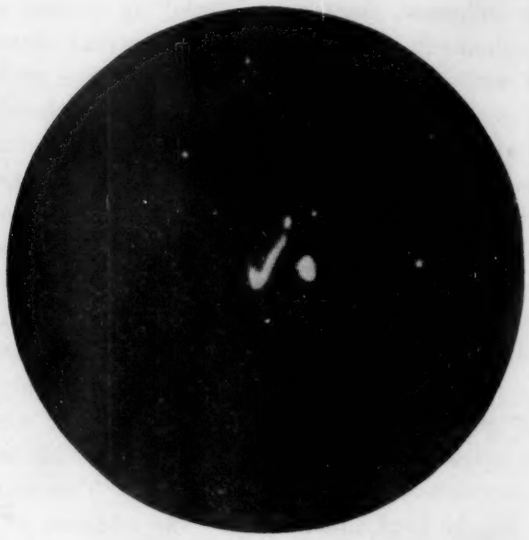

FIG. VI. Leptospira icteroides, second transfer in K medium. Beginning of granulation. Dark field illumination.

FIG. VII. Leptospira icteroides. Two weeks in K medium. Granulation complete. Organism filterable. Dark field illumination.

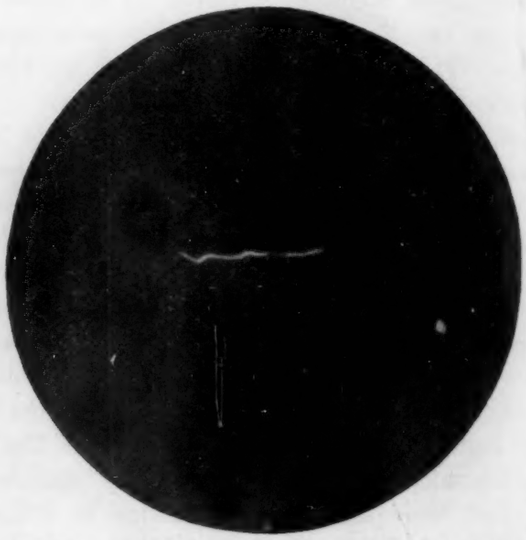

FIG. VIII. Leptospira icteroides. Growth in peptone-blood serum agar after filtration through Berkefeld N filter. Dark field illumination.

30 degrees, was productive of many small, almost invisible dew drop colonies. After transfer to blood agar (sheep), they grew as moist, fairly luxuriant white streaks. The organisms, which are members of the coccal group, appear to be somewhat pleomorphic, particularly in the first growth upon solid mediums. They tend to occur in pairs, they ferment the ordinary sugars, they are non-proteolytic, and stain with ordinary anilin dyes. They have little, if any, digestive action upon sheep hemoglobin.

Cultures No. 3Fb and No. 4Fb (in dextrose broth) remained absolutely sterile for several weeks. The coccus in the filterable state does not appear to grow in ordinary mediums, even when enriched with blood.

G. March 13, 1931. Serums from bloods No. 3Fc and No. 4Fc were filtered through Berkefeld N filters, after dilution to seven times their respective volumes with sterile physiological saline solution. One fourth cc of each serum Berkefeld filtrate was added to 10 cc of the K medium, and after incubation for 10 days at 30 degrees, was plated on the intestine-peptone agar. Colonies identical with those from cultures No. 3Fa and No. 4Fa were obtained after anaerobic incubation (3 days) followed by aerobic incubation (4 days).

Also, 5 cc of each of cultures No. 3D and No. 4D (the remainder after rabbit injections), diluted with three times their volume of sterile saline, were filtered through Berkefeld N filters. The filtrates, treated in precisely the same manner as filtrates No. 3Fc and No. 4Fc, yielded colonies which were morphologically and culturally like those had from No. 3Fa, No. 4Fa, No. 3Fc and No. 4Fc. Also, similar organisms were isolated, but more slowly, from blood No. 6 (Evanston Hospital case).

A brief recapitulation of these observations will indicate the several unusual features they present.

1. The seven influenza cases were quite similar clinically. Blood cultures made from four were sterile, even after prolonged incubation in the K medium. Three blood cultures yielded apparently identical organisms. This would seem to indicate that these organisms were not adventitious. The possibility of contamination at the time of taking the bloods is recognized, but from the nature of the organisms isolated, it is deemed rather remote, especially in light of the fact that the patients showing positive blood cultures were in two different hospitals. The four negative bloods also, from two separate hospitals, are an additional control on technique.

2. The mediums used for cultivation were autoclaved at fifteen pounds steam pressure for twenty minutes. Therefore, they were initially sterile.

3. The rabbits, both those showing symptoms (No. 3 and No. 4) and those remaining well (No. 1, No. 2, No. 5, No. 7 and No. 8), were under observation a full month before inoculation, and several weeks after inoculation. No symptoms of snuffles or other disease appeared among the control animals during this time. Three uninoculated rabbits (No. 5, No. 7 and No. 8) of the control series were kept in cages adjacent to rabbits No. 3 and No. 4, which, it will be recalled, sneezed vigorously for several days. They remained well. Apparently the infection induced in these rabbits was not very contagious for other rabbits. Blood from case No. 6, which developed turbidity in K medium much more slowly than bloods No. 3 and No. 4, was not injected into a rabbit.

4. The filters, Berkefeld N, failed to pass dextrose broth cultures of B. typhosus or the coccus above mentioned. They passed the filterable stage of the coccus readily, however.

RESULTS

From two bloods, taken from patients presenting clinical symptoms reminiscent of influenza, organisms were isolated which induced paroxysms of violent sneezing in rabbits. From the rabbit inocula, and from the blood of these rabbits, taken on the second day of infection, a filter passing "virus" was cultivated in the K medium. From the K medium, after incubation and development, cocci were isolated. These cocci, in their unfilterable state, could readily be retransformed to the filterable state upon K medium, refiltered, and recovered again, after a period of development in K medium, followed by growth on agar, as unfilterable cocci. It should be emphasized that the filterable state is readily induced by inoculation of the cocci in the special medium. The converse process, transformation of the filterable to the non-filterable state, is a relatively slow procedure. It can be done, however, by inoculation of K medium growth upon intestine-proteose-peptone agar, possibly better upon blood agar and usually, except for very fastidious microbes, as will appear later, upon plain agar. Colonies appear upon the solid mediums after a few days as tiny, clear, dew drop growths, which upon retransfer become heavier and more luxuriant. The coccus, therefore, can exist in a filterable state, in which condition it grows in the K medium, but does not appear to grow visibly in peptone mediums; and a non-filterable state, in which growth upon ordinary peptone-containing mediums is readily obtained. The rabbit infections were induced while the coccus was apparently in the filterable state. Attempts to induce infection with the non-filterable (coccus) state were unsuccessful. Experiments are under way to determine the nature and extent of changes in the virulence and infectivity of this microbe.

The relation of this coccus to the etiology of influenza naturally presents itself at this time. While the evidence, so far as it has been revealed, certainly is suggestive that more than accidental parallelism exists between the filterable state of this coccus through the rabbit syndrome to the human infection, proof is, of course, wanting. It can not be denied that possibly a filter-passing virus was simultaneously present. This might conceivably have caused the rabbit infection, and also perhaps explain the non-contagiousness by droplet infection from rabbit to rabbit. If so, it must be admitted that such an hypothetical "virus" either developed well in the K medium or else it was present in great abundance in the original blood cultures, which gave positive growths in K medium; also, if such were the case, it was also absent in the negative bloods, unless, perchance, a symbiotic action between the hypothetical "virus" and the coccus herein described was taking place.

It should be pointed out also that the dilution factor from the original blood (1:10) through cultures A–D inclusive in the K medium, would reduce the original constituents of the positive bloods many hundreds of times in the actual inocula of the rabbits. The possibility of the persistence of such a "virus" is freely admitted, nevertheless.

However, the most far-reaching phenomenon brought to light in this investigation is the obvious existence of the coccus isolated from these three cases of "flu" in two states, a filterable, and a non-filterable. Insofar as these experiments indicate, furthermore, the distinction between the two states does not depend primarily upon the somewhat academic question of porosity of filters, but upon actual, consistent differences in nutritive environment. The filterable form does not appear to develop into visible forms in plain

or dextrose broth (although occasionally they may develop upon agar under certain conditions), but it may be readily cultivated in the K medium. On the other hand, the non-filterable form, the coccus, grows readily in ordinary mediums. It changes rather readily to the filterable form, however, in K medium.

Having shown that this coccus exists in a filterable and a non-filterable state, it is not unreasonable to suspect that other bacteria might exhibit this same phenomenon of dual existence if exposed to the same cultural conditions. Such has proved to be the case.

The belief that bacteria may have a filterable state is a very old one.[5] In some instances, for example in the pus from cold abscesses, proof seems to have been procured. It is well known that formed tubercle bacilli are not found in such material, although Much granules,[6] which Fontes[7] showed could be passed unharmed through Berkefeld filters, have long been known. Not a few references to the successful passage of B. coli, B. dysenteriae (Shiga type), and B. typhosus through stone filters have also appeared from time to time in the literature, but these observations have not been very generally accepted, apparently because the experiments have not been repeatable in seriatim. On the other hand, there is a group of infections, incited by transmissible agents, capable of inducing pathological states in man, animals and plants, to which the term "filterable viruses" has been applied. The inciting agents have not, in most instances, been cultivated in artificial mediums, and little is known of the properties of these agents aside from their contagiousness, and the pathology of the lesions they incite. This embraces a large and important, but very heterogeneous group.[8] Within it are some of the most formidable infections of man. Some of the major problems in epidemiology, immunology and prevention and therapy are inextricably tied into this baffling group, whose members, resisting cultivation in ordinary mediums, have eluded science thus far.

To make this part of the investigation brief, it may be stated that the first organism examined for a filterable state was a culture of B. typhosus, which had been in stock in the laboratory for several years. It was put into the K medium, cultivated for 48 hours at 30 degrees, and after dilution with twice the volume of sterile physiological salt solution, was filtered through a new Berkefeld N filter. The clear Berkefeld filtrate was added to mediums in amounts as follows: 2 cc and 0.25 cc, respectively, to 10 cc of dextrose broth; 2 cc, 1 cc, 0.5 cc and 1 drop, respectively, to 10 cc of K medium.

Incubation of these filtrates was practiced for 48 hours in the K medium. The dextrose broth cultures remained sterile for three weeks. The 48-hour K cultures also failed to give cultures upon intestine-proteose peptone agar plates, but after 72 hours' incubation, agar cultures made from them and kept in the incubator at 30 degrees for several days, yielded numerous colonies of B. typhosus. These were characteristic morphologically and in staining reaction. Transfers of these colonies, introduced into either plain or glucose broth, agglutinated readily with specific typhoid serum.

It appears, therefore, that B. typhosus, as well as the coccus from the influenza cases, can be rendered filterable. The filterable organisms fail to grow in ordinary broth mediums, but in the K medium, growth proceeds in due course, and eventually typical typhoid bacilli may be recovered in the manner indicated. The details of control of filters and pertinent associated phenomena will appear in another communication.

Other bacteria have been similarly made filterable, filtered and recovered. To date: Rosenow's poliomyelitis streptococcus, Dochez's scarlet fever streptococcus, B. paratyphosus alpha, Noguchi's Leptospira icteroides, as well as Staphylococcus aureus, B. typhosus, and the coccus from the "flu" cases, have thus been put through their paces. It should be pointed out here, as will be explained in another communication, that not infrequently the first tiny dew drop colonies appearing on agar plates from the up growth of the filterable organisms, are very inert, both chemically and culturally. One or more transfers in ordinary mediums may be required to elicit the usual luxuriance of growth and capacity for chemical activity.

Next, it appeared that certain much discussed, not well understood phenomena, bacteriophage and antivirus, which conceivably might have a similar explanation, should be investigated. Details must be left to other communications which will follow shortly, but it may be stated here that both Staphylococcus "phage" filtrates, and Besredka "Staphylococcus antivirus" have each yielded perfectly typical cultures of Staphylococcus aureus upon cultivation in the proper manner. A sample of rabies vaccine from a lot that apparently caused a case of human encephalitis, was inoculated into the K medium. After a period of acclimatization and growth, transfer to agar plates yielded a coccus that is under investigation. In light of what has been said, it is significant that growths were not had even upon prolonged incubation in enriched peptone-containing mediums. Positive blood

[5] An excellent summary by Klieneberger: "Bakterienpleomorphismus und Bakterienentwicklungsgänge," *Erg. d. Hyg., Bakt. Immunitätsforsch. u. Exp. Therapie*, Berlin, 11: 499–555, 1920, has the important details and literature.

[6] H. Much, *Beitr. z. Klin. d. Tuberk.*, 8: 85, 1907.

[7] A. Fontes, *Centralb. f. Bakteriol.*, Abt. I. Ref., 51: 244, 1912.

[8] See T. M. Rivers, "Filterable Viruses," *J. Bacteriology*, 14: 217–257, 1927, for excellent summary.

cultures have been obtained, quite readily, from a series of cases of common cold, of arthritis, of rheumatic fever, and rheumatoid endocarditis, measles (30 hours before the appearance of the rash), and German measles. These failed under parallel conditions to yield cultures upon any artificial mediums. In one case of endocarditis, with fever, and a rather sudden increase in symptoms, an hemolytic streptococcus was isolated both directly upon blood agar (sheep) and coincidently from the K medium. It is perhaps significant that the isolation with the K medium in this instance, where the blood agar culture was positive, was completed in 72 hours. Usually thus far, when cultures in peptone mediums are negative, final isolation of the organisms from the K medium requires from 10 days to 2 or even 3 weeks. Here again, the details must be presented in another place. Enough has been related, however, to focus attention upon the apparently fundamental and general character of the phenomenon which has been described, namely, that bacteria representative of many of the important divisions of this great group of organisms can, and apparently do, exist in two distinct states. One, readily filterable through Berkefeld N filters; the other, not filterable, exhibiting characteristic morphology and staining, and with appropriate organisms, immunological reactions, each distinctive after its kind. It is perhaps unnecessary to repeat and to reemphasize here, that the multitude of details surrounding each organism necessitates separate consideration of them individually. These will appear at the proper time.

Nothing has been said thus far about the morphology of bacteria in the filterable state. It is possible to see changes which appear to lead to the filterable state by inoculating, for example, B. typhosus, into the K medium. Better and clearer preparations can be had in a "clear" K medium which can be prepared from the original medium, as will be shown elsewhere. After 15 to 18 hours' incubation in this medium, many of the typhoid bacilli, seen under the dark field, lose their homogenicity and appear first as faintly discernible shadows, having the bacillary outline, without, however, their luminous substance. They are actively motile at this time. Several brilliantly luminous, but small granules, from two to four or more, appear within the shadowy outlined organisms. The addition of specific typhoid serum to such cultivations causes agglutination, but the time required is decidedly longer than that necessary to elicit agglutination in parallel peptone broth cultures, which contain only typical bacilli. Upon retransfer and reincubation in the K medium, the bacillary forms become much smaller, and eventually for the most part are lost. Methylene blue stains made at this stage frequently reveal a multitude of very small, faintly blue staining rod-shaped bodies, enclosing more deeply stained slightly reddish granules. These shadowy bluish haloes encircling the more deeply staining central parts are reminiscent of the Rickettsia bodies, found in lice. It should be stated here, that a culture of Proteus, X19, obtained from a case of typhus several years ago, presents a very similar appearance, when cultivated in the same manner.

Finally, the bacillary part of the organism disappears, and there remain merely the very small granules, appearing under the dark field as intensely bright, yellow oval bodies. Of course the dark field furnishes no index of the true size of these granules, and but little idea of their shape. In this state, the granular vestiges of the typhoid bacillus pass an N Berkefeld filter readily. It may be recovered in the bacillary state by appropriate recultivation, as explained previously. Staphylococci similarly become smaller, and colored with methylene blue appear as oval, faintly staining haloes surrounding a granule. Upon longer incubation the nebulous halo disappears, leaving granules, which under the dark field are quite similar to those of B. typhosus. Streptococci tend to retain their linear alignment through the nebulous state, but appear to lose it when the granules are finally left as vestiges of the original organisms. Usually a proportion of the bacilli or cocci remain unchanged during the earlier stages of the process, and cultures at this period can usually be had in ordinary mediums, presumably originating from these unaltered or partly unaltered forms. Filtration, however, appears to sift these out. They remain behind, and direct cultivation of the filtered granules into non-filterable forms in ordinary plain or dextrose broth is almost always impossible. A few days' growth in the K medium, however, will almost always afford successful transit to the typical organisms. This inability of the filterable granules to develop in ordinarily favorable cultural mediums, such as plain or dextrose broth, seems to afford some evidence of the efficiency of the filters as restrainers of the normal bacillary or coccus forms of the organisms. It should be emphasized here that the mere fact that there is failure to elicit growth of the filterable forms of these bacteria in ordinary peptone-containing media is not proof they are not viable. A *small* amount of broth containing these granules, added to the K medium, will usually result in growth, even though a week or even two weeks elapses from the time of filtration to the date of recultivation. Much longer periods of survival of these granules are met with. Upon two occasions, perfectly typical strains of Staphylococcus aureus were cultivated from Staphylococcus phage which was 10 months old. Cultivation in nutrient

broth of the same composition as the phage solution itself, however, was always negative. It is of some importance to note that the coccus which was isolated from the blood of case No. 3 on February 3 was re-isolated as late as June 12. The flask containing the blood had been in the ice box for about three months. A few loopfuls were placed in K medium, and after two weeks at 30 degrees, colonies were obtained upon transfer to agar plates. Inoculations at the time into ordinary mediums were negative. Apparently the protein ingredients of the extracted intestine, which are lacking in the peptone medium in which these filterable forms exist, furnish the conditions essential for growth of the granules which seem to constitute the non-filterable state of the organism. Whether there is, or may be, multiplication of these filterable granules in phage, Besredka "antivirus" or in peptone solutions, can not be answered definitely at this time.

One rather unexpected phenomenon intruded itself during these filtration experiments, for which no explanation can at present be given. It seems to be quite in opposition to what common sense would predicate. Filtration experiments were conducted in general in accordance with this routine: the growth in the K medium, after proper development, was diluted with twice its volume of sterile, physiological saline solution, and filtered with very little vacuum; usually less than four inches below normal atmospheric pressure as measured by a vacuum gauge. The water-clear filtrate (which always showed granules, both of media, and organisms under the dark field), was distributed as follows: to dextrose or plain broth 2 cc and 0.25 cc respectively in 10 cc of medium. To the K medium, 2 cc, 1 cc, 0.5 cc, 0.25 cc and 1 drop respectively to 10 cc. Almost always, but not invariably, the tubes receiving 0.25 cc and 1 drop of filtrate from the K medium showed more rapid and better growth than those corresponding cultures containing the larger amounts of inocula from K medium. In no case thus far has growth ever been detected in the ordinary broth cultures, although sub-cultures of a few loopfuls of broth to K medium usually afforded growth eventually. This strange phenomenon has been noted so often and so consistently, it seems to be more than a coincidence. On the other hand, it seems to violate the law of diminishing returns. As an oft-repeated happening, it is proffered for what it is worth.

It is pertinent to inject here a few observations drawn from the field of bacteriology, which may have significance in light of what has been related.

Several types of infection are known in which organisms, at first demonstrable, disappear, even though symptomatology persists. Thus, in syphilis, the Treponema are clearly demonstrable in mucus patches, and in the initial lesion by dark field illumination. The organisms are rarely found in the spinal fluid in later stages, although reports of the presence of "granular bodies" in material from the cerebrospinal axis are in the literature. The ovoid bodies found by Noguchi and Flexner, and cultivated by them from cases of poliomyelitis, especially in light of the filterable nature of the material from which they were isolated, is certainly equally suggestive that these bodies are more than mere débris of media, even though current texts appear to have abandoned belief in the etiological relationship of these ovoid bodies to disease. Thus far it has been impossible to obtain an authentic culture of these ovoid bodies for study. It is very important to recall that Rosenow has repeatedly injected his streptococcus, isolated from poliomyelitis cases, into animals with the elicitation of symptoms. In many instances, especially when the animals survive for a few days before examination, it has been found difficult to demonstrate the injected organisms by staining methods, or even by culture. Reference is made at this point to the filterability of his streptococcus, of which he very courteously sent cultures, described above. It is not at all impossible to believe that the Rosenow organisms injected in the non-filterable state gradually change to the filterable state in presence of the brain substance, as previously explained. If this be true, cultivations from such animals in a K medium (or if necessary with K medium modified by substituting brain for intestine) should be successful.

Granules from cold (tuberculous) abscesses, and the appearance of Rickettsia bodies, has already been commented upon, as has the Besredka Antivirus (staphylococcus) and Staphylococcus bacteriophage. Many lesions occur in the tissues of man and of animals, in which bacteria can not be demonstrated by staining methods, and from which bacteria may or may not be isolated. It is not improbable that in many instances the same type of phenomenon of filterable and non-filterable stages of bacteria is involved in these. A culture of Leptospira icteroides (Arias strain), obtained through the courtesy of Dr. Sawyer, likewise proved filterable, thus apparently confirming Noguchi's contention that his spiral organism had a filterable stage. Blood from cases of yellow fever has not been available, consequently the rather obvious attempt to cultivate a non-filterable organism from the filterable "virus" of yellow fever has of necessity been postponed.

Granted that many bacteria, and by implication, many "filterable viruses" do have a filterable, and a non-filterable stage, the question arises, what determines the one state or the other? While a final an-

swer can not be given at this time, sufficient evidence has been gathered to formulate at least one procedure which has been uniformly successful in causing non-filterable forms of representative types of bacteria to become filterable, and after filtration, to be forced to pass back again into the non-filterable state. The phenomenon indeed appears to be quite general among the types of bacteria.

Thus far, emphasis has been laid upon the microbic side of this problem of bacterial filterability, and return to their non-filterable forms. Some important correlative information may be gleaned from a brief discussion of the conditions bacteria meet in the body itself, especially in relation to infection. Generally speaking, microbic infection of the body may be considered as taking place when the prospective invading microbe passes across the barriers which usually suffice to keep it out, and actually penetrates the protein fastness itself. A majority of bacteria, and probably a majority of "viruses" gain entrance through epithelia, principally those of the intestinal and respiratory tracts. A remarkable chemical difference between these two tracts should be emphasized here. The intestinal mucosa is almost constantly bathed in a medium rich in protein digestive products, which pass successively from highly complex peptones and albumoses to simple peptids and amino acids. The latter, so it appears, are normally absorbed through the villi of the intestine, and pass to the blood stream. Hence the mucosae of the intestinal tract are in a peptone environment, using the term "peptone" merely to indicate protein in various stages of digestion. The nutritive value of this medium is reflected in the luxuriance of the intestinal flora. The respiratory tract, and the oral cavity, on the other hand, especially the former, is by design a sterile tract. Absence of protein degradation products, except at those times when purulence is present, is the usual state of affairs. Whether mucus is of significance in this connection can not be answered in light of current information. Stated differently, the digestive tract is proteolytic, the respiratory tract is apparently aproteolytic. Bacteria that gain entrance to the digestive tract in the usual manner find abundance of protein derivatives for their nutrition, whereas bacteria that gain entrance to the respiratory tract do not normally find protein derivatives for their nutrition. Just what factors, singly and collectively, normally shield both these mucosae from bacterial invasion, are not well known, and for the moment they must be disregarded. The fact that deserves emphasis here is that bacteria within the intestinal canal are in an environment that should encourage their continued existence in the non-filterable state. Rather the contrary condition would appear to prevail in the respiratory tract. Perhaps in light of this, it is not without significance that many, if not most of the contagious so-called "filterable viruses" appear to enter, and to leave, the body through the respiratory path. Few, if any, are suspected of entering or leaving the body through the intestinal tract.

An interesting parallel is found in the observations presented above, namely, that the filterable state of bacteria can apparently be induced by cultivating them in the K medium, which by design is rich in substance approaching tissue protein in composition, and free from peptones and most alcohol soluble nitrogenous extractives. Bacteria that pass from the respiratory tube actually into the tissue of the lung, whether they are filterable or not as they enter the respiratory tract, become confronted with a protein-rich peptone-poor medium. From what has been stated, this is one condition which tends to induce and perpetuate the filterable state. Similarly, upon passing back from the lung tissue to the respiratory tube, the same condition prevails, unless there is purulence, which, as is well known, is associated with protein digestion. Therefore, filterable states of bacteria should theoretically be not uncommon in infections of the respiratory tract, in absence, of course, of pus formation. Perhaps it is unnecessary to reiterate that many, if not most of the "filterable viruses" are found in association with the respiratory, rather than the intestinal tract. Bacteria leaving the body through the intestinal tract are exposed to nutritive conditions conducive to the non-filterable, rather than the filterable state. Such appears to be the case in light of current information. This is not the whole story, however. The fact that bacteria may pass readily into the filterable state does not explain just why bacteria, even in this state, can induce infection. Colon bacilli and typhoid bacilli, as has been shown above, are readily filterable, when grown in presence of protein and absence of peptone, but colon bacilli do not, except relatively rarely, invade the body. The nature of the weapons with which certain kinds of bacteria force entrance through intact epithelia, which ordinarily suffice to keep microbes out, is still to be determined.

Not enough work has been done to date to be dogmatic, but sufficient experimental evidence has accumulated to show quite clearly that the bacteria studied can be made to pass rapidly and readily to the filterable state by cultivation in the K (protein) medium, and what is perhaps equally significant, the converse is true, because several successful cultivations of bacteria of various types have been made from the blood stream in cases of influenza, common cold, arthritis, rheumatic fever, measles, and other

pathological states, where bacteria have for a long time been confidently expected to exist, but from which cultivations in artificial mediums thus far have been rather regularly negative. Applying the same reasoning with experience gained with this K (protein) medium to infections in the body, it seems not unreasonable to believe that as soon as bacteria as they are known in culture (the non-filterable state) pass into the tissues through the barriers that ordinarily suffice to keep them from the tissues, they are in a protein environment, which is free, or nearly so, from peptones and similar products of protein disintegration. Under these conditions, bacteria may be expected to change from the non-filterable to the filterable state. In this state their migration from place to place might reasonably be facilitated, and what is equally significant, their existence in the granular or a filterable state would probably explain why it is that bacteria as such are so infrequently demonstrable by stain or by culture in the body tissues except where pus collects. Pus is rich in degraded protein. Possibly an interesting exception may be leprosy, where, as is well known, large numbers of stainable bacilli may be found in nodules devoid of evidence of local proteolysis. In arthritis, and many other long drawn out microbic processes, culture and stain alike have usually failed to furnish unequivocal evidence of the presence of microbes, yet in the few cases studied in this series, using a medium adapted to the growth of these filterable forms, bacteria have been grown, where in the past sterility has been the rule. It should be emphasized that even in these cases, referred to above, cultivations in the ordinary mediums, even enriched with blood, have usually been wholly in vain. The objection may well be raised that the successful cultivation of organisms from a very few such cases mentioned above is not necessarily an indication of etiological relationship. Bacteria might be present more or less normally in the blood stream. It may be remarked, parenthetically, that if such be the case, the fact is well worth further investigation. Normal tissues from animals are notoriously bacteria infested. In this connection also it may be pointed out that organisms have been cultivated from two breast carcinomas rich in lymphoid tissue, and one lymph gland adjacent to a rectal carcinoma. In these it would appear reasonable to suspect however that the regional lymph glands have merely strained out the bacteria that happened to be brought to them from time to time. Even tubercle bacilli have been found in lymph glands without evidence of infection.[9] However, bloods taken from nine normal persons have been most carefully examined as controls upon this point. They were all free from cultivable bacteria.

The problem of immunity, in light of these observations, takes on a new aspect. On the one hand, the beneficial effects reported during the use of phage and Besredka Antivirus for therapeutic purposes would seem to be related, not to enzymes or toxins, but rather to the presence of viable, filterable stages of bacteria, although, of course, enzymic and toxic effects are not disproven by any means. Experience with vaccine virus and rabies vaccine, on the other hand, shows very clearly the protective efficacy of living, but (thus far) uncultivable "viruses," whose virulence has been purposefully profoundly altered by growth in other animals for sufficient time to change the host infectiveness. Suggestions of the mechanism of alterations in host infectibility seem to be foreshadowed in some of the filterable forms of bacteria studied in this series. It has repeatedly been noted, for instance, that filterable typhoid bacilli, cultivated serially in K medium prepared from hog intestine, grow scantily and slowly for several transfers in corresponding K mediums prepared in the same manner from dog or rabbit intestine. Later, when culture acclimatization has taken place, it is rather difficult to pass the cultures in the filterable state back to hog intestine medium again. A more rapid procedure of adaptation is to start with the typical, non-filterable typhoid bacillus, and inoculate directly from ordinary mediums to hog, dog, or rabbit intestine mediums as desired. This is perhaps a cultural analogue to the well-known passage of human smallpox virus through a series of heifers to the vaccine virus state, or the passage of dog rabies through rabbits to the virus fixé state. However, it should also be noted that some bacteria which grow fairly readily in a hog intestine K medium grow badly, or even not at all in dog intestine, and vice versa.

The relation of the filterable forms of bacteria to phagocytosis, and to serologic immunity, have thus far not been studied at all. A large amount of work will have to be done to cover the multitude of problems that immediately arise from this dual stage of microbic existence.

A word of caution must be interjected. It is absolutely necessary to be assured of sterile mediums. A double autoclaving has been found satisfactory for the K medium, but this can not be practiced with egg white. It coagulates. Egg white, filtered through Berkefeld W filters (after dilution with sterile physiological saline solution) is rarely sterile. Bacterial growth can usually be obtained from such egg white filtrates, although otherwise, fresh egg white filtered and diluted with Tyrode solution is a rather good

[9] See Trudeau and Krause: *J. Med. Research*, 22: 277, 1910.

medium for the elicitation of filterable forms of bacteria. This is again evidence, it would seem, of the existence of filterable, viable forms of bacteria in purely protein solutions. And not only must mediums for the cultivation of the filterable states of bacteria be sterile; material from the human or animal body, and even cultures of bacteria themselves, in light of what has been stated, must be thought of as having possibly two or more distinct organisms living side by side. It has long been known, for example, that the virus of foot and mouth disease can be carried along with vaccine virus for considerable periods, and there is therefore the possibility that blood cultures may harbor more than one organism. In the foot and mouth disease-vaccine association, and in the filterable virus group generally, the protein which is usually associated, either from the body or in the artificially enriched medium, might suffice to keep the viruses in the filterable state, in which instance their presence would be readily overlooked. The details of recognition and separation of such complexes will naturally vary with individual cases.

Bacteria also may be similarly associated: there is some evidence already indicating that a filterable state of one microbe may be actually carried along, unrecognized, with a stock culture of another microbe, especially if transfers are made upon plain, nutritive meat infusion agar. One form may remain hidden, and entirely unsuspected until cultivation is practiced for a period of time in K medium. Reestablishment of the non-filterable form may reveal two organisms, where only one was presumed to be present.

Finally, a word about mediums. It is very obvious that the K medium, even with its variants as suggested above, is by no means the best that may be concocted. The same tissue from different animals affords, in specific instances, some differential advantages for specific cultivation of one or another microbe; therefore different mediums should be available. Reasoning from analogy, with some experimental evidence also, it would seem to be consistent to utilize homologous tissue from homologous species for difficult isolations. Also, within limits which vary with the type of microbe, there is advantage in training them to develop in alien host tissue protein for purposes of immunization and experimental infection.

SUMMARY AND CONCLUSIONS

1. The isolation of a filter-passing diplococcus from the blood of certain cases of influenza by means of a special cultural medium is described. The experimental effects of this organism, while in the filterable state, upon rabbits, is discussed.

2. A procedure is formulated for inducing at will both a filterable and a non-filterable state in bacteria. Mention is made of a series of experiments in which both the filterable and the non-filterable state has thus been induced in a series of well-known bacteria comprising a variety of types.

3. It is postulated that a majority, if not all, known bacteria can and do exist in a filterable and in a non-filterable state.

4. A preliminary report of the isolation of microbes in the blood, not only of cases of influenza, but also from common cold, rheumatic fever, arthritis, from Staphylococcus bacteriophage and Besredka's Staphylococcus Antivirus is presented in evidence of the ubiquity of the procedure.

5. An explanation of the chemical basis for the existence of bacteria, both in the filterable and non-filterable states, in the animal and human body, and in culture, is proffered.

6. The relation of this chemical concept to microbic infection, and the state of microbes in the body during infection is discussed.

In conclusion, it is a privilege as well as a pleasure to inscribe here my appreciation for the courteous cooperation of Dean Irving S. Cutter, Doctors Charles A. Elliott, Paul Starr, James G. Carr, Walter Nadler, Howard Alt and Herbert Barker, of the department of medicine, and to Northwestern University for the generous facilities and unrestricted opportunity for research which have contributed immeasurably to this investigation.

ARREST OF GEOLOGIC, ARCHEOLOGIC AND PALEONTOLOGIC WORK IN CENTRAL ASIA

By President HENRY FAIRFIELD OSBORN

AMERICAN MUSEUM OF NATURAL HISTORY

A PEIPING society originally known as the Cultural Society, but now more definitely organized as the Commission for the Preservation of Antiquities, on June 3, 1931, addressed the following letter to Dr. Roy Chapman Andrews in reply to an application of May 5, 1931, for the continuation of the American Museum explorations and researches in Mongolia:

Sir:

We beg leave to acknowledge the receipt day before yesterday of your note stating that members of your

Museum desire to proceed to Mongolia next year to resume their work of geological research.

In discussions held at meetings of the Committee, all present have considered that, since your expedition repeatedly declared, at the time when you demanded permission to proceed to Mongolia to carry on your work last year that the expedition was going to wind up its past work and would therefore discontinue its trips, and since the Chinese Government has now organized a Western Frontier Scientific Expedition to proceed to Mongolia, Kansu and Sinkiang to carry on various scientific researches itself, during the present period of time there of course exists no necessity for permitting members of your Museum to make further trips to carry on this work.

Should American scholars be willing to come to Peking to make scientific research into the geological materials concerning Mongolia to be obtained in future as a result of surveys by members of the Chinese expedition, the Chinese Government will certainly afford them all facilities in order to conform with the principles of non-discrimination between nationalities in respect of science.

The restrictions imposed by this society have not been placed on American exploration alone, for somewhat similar actions have been taken against the Swedish Expedition in Chinese Turkestan under Sven Hedin and more recently against the French Trans-Asiatic Expedition under Dr. Georges Haardt and P. Teilhard de Chardin, distinguished paleontologist and geologist, and the British Expedition under Sir Aurel Stein. This is in line with the now openly professed policy of the Peiping Commission to stop all foreign scientific work in China, no matter by what nationality. They have driven the distinguished archeologist, Sir Aurel Stein, out of Chinese Turkestan; they made very severe conditions for Dr. Sven Hedin on his last geographic expedition to Chinese Turkestan. They are putting every obstacle in the way of the present Trans-Asiatic Citroen Expedition of the French; they now bring to a full stop the ten years' work of the Central Asiatic Expedition of the American Museum.

In the meantime by articles in the Chinese newspapers this commission is giving out erroneous statements as to the work of these various foreign explorations, rousing a hostile attitude among the people and thereby cultivating among the Chinese the thought that all foreigners are enemies of their country, and it is surprising to find that the membership of this commission is not confined to Chinese who are ignorant of the friendly and sincere intentions of the United States towards the Chinese people and their institutions, but that it includes returned American students who should understand the American attitude toward China and be grateful for it.

For three years past, namely, since August, 1928, when the Peiping Commission seized the Mongolian collections of the Central Asiatic Expedition at Kalgan the American Museum has been working through the Chinese Minister, Dr. C. C. Wu, through Secretary of State Stimson, through Assistant Secretary Nelson T. Johnson, now Ambassador to China, and through the Chinese Government of Nanking to overcome the hostility of the Peiping Commission and set before the Chinese the true state of affairs, namely, that throughout its entire ten years of exploration in China and Mongolia, the American Museum of Natural History has been actuated only by the highest and most generous motives. Every stage of its procedure has been with the full knowledge of the Chinese Government at Nanking and Peiping and with the Mongol Government at Urga—not only with full knowledge but with full prior consent. Moreover, it was agreed from the outset that the American Museum would leave entirely untouched the tempting fossil fields within the boundaries of China proper to the well-organized National Geological Survey of China, and that it would cooperate with the National Geological Survey of China in sending duplicates or replicas of fossils not only of Asiatic origin, but of American fossils cognate to those to be discovered in Central Asia. More than this, during President Osborn's visit to Peiping in the year 1923, he offered to establish a branch of the American Museum within one of the now vacant palaces of the Forbidden City, to send duplicates of all the zoological collections made in China and all the zoological and paleontological collections made in Mongolia. The then director of the Peiping Museum of Art, Dr. Kungpa King, took the deepest interest in this project and actually aided in the selection of one of the palace buildings suitable for the exhibition of natural history collections. Long prior to this the American Museum issued a manifesto which was widely printed and circulated throughout northern China declaring its sympathy in the preservation of all archeological and prehistoric objects and its opposition to looting and destroying these precious records of the ancient history of China.

The abrupt interruption of these friendly and most promising relations came with the seizure of the entire collections of the year 1928 by the organization then known as the Cultural Society. Long months of negotiation between the American Museum, Washington, Nanking and Peiping ensued, culminating in the final release of this collection of fossils obtained entirely within the borders of Mongolia and beyond the confines of China, and composed almost entirely of geological and paleontological objects. In the year 1929 permission to reenter Mongolia was again refused by the Peiping Commission; in the year 1930 the permission was reluctantly granted on the understanding that the Central Asiatic Expeditions would

terminate. This was followed by the discovery of entirely new fossil fields of Pliocene age to the east of the Kalgan Urga Trail; it was this discovery which led the American Museum to request a renewal for the present year 1931 and for the coming year 1932.

At this point a brief recital, by the leaders Andrews and Granger, of what has been accomplished by the American Museum may be given:

Dr. Roy Chapman Andrews has been unable to make arrangements with the Commission for the Preservation of Antiquities of Peking, China, for the continuation of explorations in the Gobi Desert by the Central Asiatic Expedition. For ten years this Expedition, under the auspices of the American Museum of Natural History, New York, has been making a general scientific survey of Mongolia, partly with the hope of finding the birthplace of the human race. Its researches have opened a new volume in the history of world life. It has spent half a million dollars in the country and has in every way conformed to local customs and restrictions. When its work was first started in 1921 it entered into an agreement with the National Geological Survey of China in which it was expressly stated that the Expedition was free to work in Mongolia. The terms of this agreement have been scrupulously kept by the Expedition.

The Central Asiatic Expedition was organized in 1920 and the advance guard (Messrs. Andrews, Granger and Pope) reached Peking and established headquarters there early in 1921. Since then the Expedition has made five summer explorations in the Gobi Desert (1922, 1923, 1925, 1928 and 1930). The only paleontological work done outside of Mongolia was three winters' work (1921–1922, 1922–1923 and 1925–1926) by Mr. Granger, at Wanhsien, a locality which was turned over to the Central Asiatic Expedition by the Geological Survey of China because they could not, on account of disturbed political conditions, investigate it themselves. The only archeological work done by the Expedition outside Mongolia has been an investigation by Dr. Nelson (1925–1926 and 1926–1927) of the Neolithic Culture found along the banks of the upper Yangtse River.

In 1928 our Expedition went to Mongolia under permission obtained for us by the American Minister from Chang-Tso-Lin, who was then in command of Peking. Our first contact with the Cultural Society came in the autumn of that year when we attempted to bring back to Peking our season's collection. The transport of our cases was blocked at Kalgan and it required two months of negotiations and the payment of a ransom collection of American fossils to finally get them free. In 1929 the Expedition was prevented from taking the field because of our inability to meet the demands of the Society. In 1930 these demands were modified to such an extent that the Expedition found it possible to accept them and our fifth Gobi exploration was carried out. It was agreed that year that this would be our last expedition but because of the important Pliocene deposits discovered in the Eastern Gobi it was desired to continue for one or two years more in the hope of achieving the chief aim of the Expedition, *i.e.*, the discovery of remains of ancient man.

Points to be emphasized are the following:

(1) Not a single specimen of any historical or archeological value, aside from Neolithic flints, has ever been taken from China or Mongolia by the Expedition, and these flints are still to be found strewn over the surface of the Gobi in millions.

(2) The great majority of fossils obtained have come from Outer Mongolia—a territory over which China relinquished the last vestige of control before our first trip there in 1922.

(3) It was arranged at the beginning of the Expedition that duplicates of all fossils would be returned to the Chinese Geological Survey. This arrangement has been lived up to and already the Survey Museum has been much enriched by duplicate specimens thus returned, and eventually there will be in China a very adequate representation of the collections made by the Expedition in Mongolia. Also, examples of all types of Neolithic flints have been given to the Survey Museum by Nelson.

(4) The Central Asiatic Expedition has always paid its way—spending a very considerable sum of money in China, also it has always worked openly, putting its cards on the table before each trip and at the close of the season announcing in public meetings in Peking (called usually by the Paleontological Society of China and held in the Survey Library), and in the press, the full results of the season's work.

(5) Those of us who have been associated with this Expedition can not help but feel that the presence of the Central Asiatic Expedition in China, with its personnel of talented men, has been an inspiration to Chinese scientists, and that the Expedition has been an incentive to the Chinese to do something for themselves along the same lines. In other words, we feel that our presence in China has been a benefit rather than a detriment to the country as a whole.

(6) The Expedition has not looted! It went into China in 1921 with the approval and cordial assistance of the Chinese scientists in Peking and their cordiality continued until 1927 when the self-constituted body, calling itself the Cultural Society, came on the scene and proceeded, by false representations, to turn the public against us and eventually the scientists of China, in self-defense, have had to follow suit.

The principal discoveries of the Central Asiatic Expedition are as follows:

(1) The discovery in 1922, and the development (1922–1930) of one of the world's great fossil fields, with no less than twelve distinct faunas—Lower Cretaceous to Pleistocene.

(2) The discovery of dinosaur egg nests, and of the complete skeletons and remarkable growth series of skulls of the animals which laid the eggs.

(3) Discovery of Cretaceous mammal skulls and associated parts of skeletons. The only Mesozoic mammal skulls known except *Tritylodon* from South Africa.

(4) Much additional information regarding *Baluchitherium*, the world's largest land mammal.

(5) Discovery of *Embolotherium*, a new phylum of Titanotheres and the most unusual and distinctive animal discovered by the Central Asiatic Expedition. The extraordinary development of the nasals in this creature is unparalleled in modern or extinct forms.

(6) Discovery of new phyla of Amblypoda and the extension of this order to mid-Oligocene time.

(7) Discovery of a great deposit of the shovel-tusk Mastodon, *Platybelodon*, with a remarkable series of specimens showing growth stages from foetal young to old age.

CONCLUSIONS

The arrest of British, French, Swedish and American paleontologic and archeologic work in Central Asia constitutes a very serious setback to the cause of science and of civilization. The matter would not be so serious if there were any possibility or prospect of the present ability of the Chinese to carry out this work themselves. They have neither the scholarship nor the financial means of doing so beyond the confines of old China. The American Museum geologic, paleontologic and stratigraphic and topographic work in Mongolia and the great publications issuing therefrom have been possible only because the party was composed of a body of field experts such as has never been brought together before in the history of these branches of science, under a leader who has shown unprecedented ability to organize a series of expeditions into an absolutely unknown desert where all previous explorers had failed either to make discoveries or obtain substantial results.

Our first knowledge of the paleontology of China came from fossils purchased in apothecary shops; for hundreds, perhaps thousands, of years fossils have been collected in various parts of China and ground up as medicine under the belief that they represent the remains of dragons. The American Museum expeditions have been among the first to dispel this nation-wide superstition, and American institutions of medicine have been advancing the true art of medicine throughout China. It is these very fossils, valueless in themselves, priceless in the knowledge they yield of the past history of the earth, the collection of which the Peiping Commission is now arresting.

The friendly effort of the American Museum to train up a body of young Chinese who would establish the extremely difficult sciences of field geology, vertebrate paleontology and prehistoric archeology under the auspices of the proposed Natural History Museum of Peiping has been brought to a full stop by the ignorance and anti-American prejudice of the Society for the Preservation of Antiquities.

This arrest of Central Asiatic exploration and research will cause world-wide disappointment and regret, especially among those who have been sincerely desirous of soundly establishing these great branches of science in China. The Commission for the Preservation of Antiquities must, therefore, bear a heavy weight of responsibility for the retardation and finally for the arrest of scientific researches and explorations in Central Asia, whereby China is placed in the column of backward, reactionary and non-progressive nations.

OBITUARY

ALDRED SCOTT WARTHIN

ALDRED SCOTT WARTHIN, student of disease, came into medicine by way of music. An artist he remained throughout life in the intensity and the individuality of his impressions. It was fitting that his first research should deal with the physiological effects of music and his last with the coming of death to the physician as depicted in art. His work on morbid anatomy, clinical medicine and experimental pathology was done with a sensitive recognition of the actual, but from it he derived convictions that were passionately held.

Born in Greensburg, Indiana, in 1866, Dr. Warthin gained a teacher's diploma at the Cincinnati Conservatory of Music in 1887, an A.B. at the University of Indiana in 1888, and graduated in medicine at the University of Michigan in 1893, becoming doctor of philosophy by the way. He was organist in churches to help himself through. The next four years he spent in the study and teaching of internal medicine at Michigan, with some months in Vienna and Freiberg. Then, turning his abilities to pathology, he became within six years Professor and Director of the Pathological Laboratory, taking his place amongst a brilliant faculty. There he remained, happily striving with the opportunities created by his diverse talents even more than provided for them, until his death on May 23, 1931. In 1900 he married Katharine Angell, herself a physician. He was made doctor of laws by the University of Indiana in 1928.

Dr. Warthin early decided that morbid anatomy was not the worked-out lode that many deemed it. Nor was it for him. The material at his disposal then and for some years after was meager, but for this he more than compensated by an intensive scrutiny. Rare was the post-mortem examination at that time to which he did not give three entire days. And overlooking nothing, finding a theme even in the pathology of the pacinian corpuscle, he became aware of the small signs of things that mattered largely.

This is most noteworthy in the long series of his discoveries with regard to lurking syphilis; but it is no less evident in the studies on the lymphoblastomatous conditions, on thymic hyperplasia and the status lymphaticus, on the changes produced by the Roentgen rays and on the significance of the hemolymph nodes, those little structures which had seemed so debatable until he proved them to be organs in their own right. It was in relation to syphilis, though, that he rendered chief service as investigator. By discriminating techniques and pertinacious observations he disclosed this disease in what had been taken always for innocent conditions, proving that it still tricks the doctor who for generations has been warned of its tricks.

Contributions of other kinds he made in great variety and number. A forceful, interested teacher whose influence continually ramified during forty years, Dr. Warthin met country-wide demands for lectures. He wrote text-books and comprehensive articles, labored as editor, served on public health committees, assumed presidencies, gave himself in short to activities which he knew to be alien to his main purposes, but carried on because he felt their need. In scientific meetings he spoke with a candor wholesome in this day when errors of fact during public discussions in the search for truth are too often condoned or reserved for hole-in-the-corner criticism. He expected as much of his fellows and experienced some naïve disappointments. As a humanitarian he would have been too aware of the dark side of life for his own good or that of others had not the bright side affected him still more. He could turn from a gruelling conference on venereal disease to his garden, or to music, to medical history, or merely to the consciousness of existing vigorously, in which he took great delight.

Possessed of a singular gusto for life yet utilizing his strength for the comprehension of the morbid, Dr. Warthin was drawn latterly to studies of the aging process and of some aspects of death itself. His book, "Old Age, the Major Involution," deals with the physical changes which constitute senility. It was characteristic of him that he spared no material detail yet had recourse in the end to the imagery of the Book of Ecclesiastes. In the "Creed of a Biologist" he set forth the view, as had Condorcet in the imminence of death, that it should suffice for man to believe in the progress of his kind and to make efforts toward it.

Dr. Warthin's last book, "The Physician of the Dance of Death," is in its immediate aspects an analysis of manners. During many years he had collected "Danses Macabres" in almost unrivaled variety, and the little volumes, peopled with the men and women of four centuries, all suddenly aware that they are to die, furnished material to his hand. From amongst these he selected only the doctor, treating his theme in a matter-of-fact way like so much else that, thus handled, had yielded its secret. The first copies of the new-printed book awaited signature when death came to him—unrecognized, after all, since he saw in angina pectoris only a familiar asthma.

PEYTON ROUS

THE ROCKEFELLER INSTITUTE

MEMORIALS

THE *British Medical Journal* states that a meeting of the Osler Club was held in London on July 12 to celebrate the eighty-second anniversary of Sir William Osler's birthday. Dr. William Stobie, Mayor of Oxford, delivered the fourth Oslerian oration. Dr. A. Salusbury MacNalty, of the Ministry of Health, proposed and Dr. A. P. Cawadias seconded, a vote of thanks, and Sir Percival Hartley briefly spoke in the discussion. Among those present were Sir D'Arcy Power, Dr. J. D. Rolleston, Dr. K. R. Hay, Professor D. Fraser-Harris, and Mr. Arnold M. Muirhead, whose recent "Memoir" of Lady Osler was displayed among the exhibits.

A BRONZE bust of the late Professor Clemens Pirquet was recently unveiled in the garden of the New General Hospital of Vienna, when addresses were delivered by Professors Argt, Hamburger and Noeggerath, and Miss Harriette Chick, of the Lister Institute.

RECENT DEATHS

DR. RICHARD ALEXANDER FULLERTON PENROSE, JR., until his retirement in 1911 professor of geology at the University of Chicago, died in Philadelphia on July 31, at the age of sixty-eight years.

THE REVEREND JOHN BERNARD GOESSE, S.J., professor emeritus of geophysical observations at the Saint Louis University, died at Saint Louis, July 25, at the age of sixty-two years. Father Goesse was the founder in 1908 and the first director of the Geophysical Observatory of Saint Louis University and took a prominent part in the organization of the first Jesuit Seismological Service in 1909, together with Father Odenbach, of Cleveland, and Father Tondorf, of Georgetown. His early retirement had been due to prolonged ill health.

DR. ERROL LIONEL FOX, professor of chemistry at Washington College, Chestertown, Maryland, died on July 17 in Munich, in his thirty-ninth year.

DR. WILLIAM C. HASSLER, who served for thirty years as public health officer of San Francisco, died on August 2. Dr. Hassler was elected this year president of the American Public Health Association.

DR. EDWARD L. CREEDEN, head of the Bureau of Preventable Diseases of the New York City Department of Health and a member of the department for twenty-five years, died on July 31. He was fifty-five years old.

PROFESSOR EMIL WARBURG, eminent physicist, formerly president of the Federal Physical-Technical Institute and a member of the Prussian Academy of Sciences, died on August 1. He was eighty-five years old.

SCIENTIFIC EVENTS

THE JUBILEE MEETING OF THE BRITISH SOCIETY OF CHEMICAL INDUSTRY

THE celebration of the jubilee of the Society of Chemical Industry, held in London during the second week in July, is described in the London *Times*. It included a reception at Guildhall by the Lord Mayor and Sheriffs; an address by the president, Sir Harry McGowan, at the Royal Academy of Music, and receptions by the Masters of the Girdlers' and the Salters' Companies. The presentation of the society's medal to Dr. Herbert Levinstein was made on July 15, and in the evening the annual dinner was held at which Prince George was the principal guest. The following day was devoted to the discussion of technical papers. There were visits to works and excursions in and near London.

The society was actually established in London, in the rooms of the Chemical Society early in April, 1881, with Professor (afterwards Sir Henry) Roscoe in the chair. Most of the forty-nine annual meetings have been held in various cities and towns in Great Britain, but three have been in New York and one in Montreal. Similarly, many active local sections have been formed, not only in England but also in Canada, Australia and the United States. The society, which received a Royal Charter in 1907, has now over 7,000 members.

As a small token of its admiration for work, the society presented a brief address to a few of the companies which had been most prominent in their support of technical and scientific education, and to a few whose work had been specially directed to those branches of education in which the chemical industry was specially interested. Plaques were also presented to many original members of the society and to a number of past presidents.

Throughout the week an exhibition of chemical plant, arranged by the British Chemical Plant Manufacturer's Association and the Chemical Engineering Group of the society, was open at the Central Hall, Westminster. The association was formed in 1920 to further the production and use of chemical plant made in this country. Over 50 British firms have cooperated to display the range and variety of chemical apparatus which their works can turn out. Where possible the actual plant was on view, sometimes in operation, but apparatus too large for exhibition was illustrated by models or photographs. In some cases cinematograph films were used to show processes of manufacture.

To illustrate the application of chemical processes to manufacturing industries generally would, however, have required very much more space than was available, and therefore chemical plant in the narrower sense bulked most largely in the exhibition. There are many exhibits of acid-resisting stoneware, porcelain, fused quartz and protective linings of various kinds as well as of the resistant alloys of steel and aluminium which have been developed of recent years, of nickel, and even of silver, which at present prices is becoming a possible material for some purposes. The exhibits also included pumps, filters, centrifugal separators, drying machines, thermometers, and other measuring instruments, and the absorbent substance, silica gel, which contains such large numbers of minute pores that a cubic inch has been calculated to possess an internal surface of over an acre.

A separate section of the exhibition, organized by the Chemical Engineering Group of the Society of Chemical Industry with the assistance of the Department of Scientific and Industrial Research and the Research Associations of various industries was designed to illustrate the application of scientific research to industry. It included materials used in chemical engineering, such as metals, fabrics, rubber, leather and paints, chemical plant, especially that applicable to fuel, and methods of testing and standardizing apparatus and materials.

THE BUCKSTON BROWNE SURGICAL RESEARCH FARM

THE foundation stone of the Buckston Browne Surgical Research Farm at Down, near Farnborough, Kent, was laid by Lord Moynihan, president of the Royal College of Surgeons of England, on July 8. The site is one of thirteen acres, adjoining Down House, Charles Darwin's old home, which, with its grounds of twenty-three acres, was presented by Mr. Buckston Browne to the British Association for the Advancement of Science.

According to the *British Medical Journal*, the stone which has been laid will form part of the porch of the large residential building, in the style of a Kentish farmhouse, which will house the research workers, as

well as those in charge of the laboratories and the animals. The building of the animal houses and the experimental laboratories has not yet begun. A brief service of dedication was held by the dean of Westminster, who, after Lord Moynihan had laid the stone in place, pronounced the words: "Here may knowledge be increased; study fostered; skill developed; to the prevention and relief of suffering, the service of our fellow men, and the good of the human race throughout the world."

Lord Moynihan made the principal address. He said in part:

> The Council of the Royal College of Surgeons, which properly regards itself as the custodian of surgical advance in this country, has now recognized that for the further expansion of surgery there must be a correlation of the various results from methods of inquiry in three directions: (1) clinical research carried out also in the wards and in the operation theaters of hospitals; (2) biological and pathological research carried out in hospitals and in special institutions, and (3) research upon animals. In accordance with the council's will, laboratories have been built and old laboratories extended in our buildings in Lincoln's Inn Fields; there men have been set to work upon lines which they themselves desire, and there men have learned "the religion of research," and the high value of the experimental method in connection with the science and art of surgery. Now, owing to the great beneficence of a fellow of our college, we are here laying to-day the foundation stone of an institute for experimental research, which will add the one remaining event required for the proper development of surgery in this country.

At the conclusion of this address Mr. Buckston Browne spoke briefly, calling attention to the "very interesting fact that they had been able to bring the great genius of John Hunter, who did so much for the alleviation of the suffering of the human body, alongside that great genius Charles Darwin, the emancipator—as I like to think of him—of the human mind. These two great men are brought together to-day on this really sacred spot in Kent."

After tea had been taken, the company made their way along the sand walk—Darwin's "thinking path"—to Down House, the property which was presented in 1927 by Mr. Buckston Browne to the British Association as a memorial of Charles Darwin, where they visited Darwin's study, kept as it was during his lifetime, with the little paraffin lamp on the table, and the dog (now a trophy of the art of the taxidermist) in its basket on the hearth.

THE SIXTH INTERNATIONAL CONGRESS OF GENETICS

THE sixth International Congress of Genetics will meet in Ithaca, New York, from August 24 to 31, under the presidency of Dr. T. H. Morgan, director of the William G. Kerckhoff Laboratories of the Biological Sciences at the California Institute of Technology.

The New York entertainment committee, of which Dr. A. F. Blakeslee, of Cold Spring Harbor, is chairman, has completed a tentative program for the four days from August 20 to 23, intervening between the arrival of the *Pennland* and the departure of the delegates for Ithaca. The Eugenics Congress is in session in New York at this time and many of the delegates will desire to attend some of the sessions. During their four-day stay foreign members of both congresses will be entertained at Columbia University, through the cooperation of the university and the Carnegie Endowment for International Peace. Rooms will be provided in the Columbia dormitories, and breakfast will be served at the Faculty House.

According to the tentative program the scientific laboratories of Columbia University will be visited on August 20, after which a luncheon will be given. This will be followed by a sight-seeing trip around the city. At 7:30 p. m. there will be a formal complimentary dinner. During the time spent in New York there will be an excursion to Cold Spring Harbor to visit the Carnegie Station for Experimental Evolution and other scientific laboratories at Cold Spring Harbor, visits to the New York Botanical Garden, the Boyce Thompson Institute, the Cornell Medical School, the Rockefeller Institute and the Medical Center of Columbia University. One evening will be devoted to the addresses and reception of the Eugenics Congress at the American Museum of Natural History.

On August 23 there will be a trip to Princeton, members of the congress being the guests of the Walker-Gordon Laboratories. For those not taking the Princeton trip an opportunity will be given to visit the American Museum of Natural History, the Metropolitan Museum of Art, and other places of interest in the city, with guides for groups if desired. Dr. Stockard has offered to guide a group to his dog farm near Peekskill and Dr. Davenport a group to the State Institution for Feeble Minded at Letchworth Village. Luncheon will be at the American Museum of Natural History and in the evening a smoker or informal reception will be given.

Tentative arrangements have been completed at Ithaca for a comprehensive exhibit of genetic material. The Carnegie Corporation of New York has appropriated $5,000 for the general expenses of the congress. A grant of $2,000 has been made by the Carnegie Institution of Washington for the Genetics and Eugenics Congresses, the sum to be used pri-

marily for the exhibit. Of this grant the Genetics Congress received one half.

It is planned to have an excursion to Niagara Falls during the Congress. The cost of a round trip on a special train is about $3.00 per person. An excursion is also planned during the congress to the New York Agricultural Experiment Station at Geneva (about 50 miles north of Ithaca) where there will be exhibits dealing with fruit genetics and fruit breeding. Meetings of sections, interested in fruit work will be held at Geneva during this excursion.

It is expected that after the congress members from abroad will wish to take short trips in order to gain some impression of the scenery, the colleges, universities, public institutions and agriculture of the parts of the United States which are easily reached from Ithaca. Arrangements for transportation and entertainment for two organized excursions will be made—one through southern New England, including visits to Harvard, Yale and other colleges, Woods Hole, and to farms and agricultural experiment stations; and one to Washington, including stops at other cities and universities *en route*. For those members who wish to take longer trips to the west and south the committee will be glad to provide information and help, but because of the greater time and expense involved, no organized excursions will be planned unless a large number of persons request it.

SCIENTIFIC NOTES AND NEWS

MME. MARIE CURIE received the gold medal and honorary fellowship of the American College of Radiology at a luncheon given in her honor at the third International Congress of Radiology, which opened at the Sorbonne, Paris, on July 26. The presentation was made by Dr. Albert Soiland, of Los Angeles.

DR. CHARLES L. PARSONS, of Washington, D. C., long secretary of the American Chemical Society, has been elected to honorary membership in the Society of Chemical Industry, the insignia of this distinction having been presented by Prince George at the jubilee meeting of the society in London. Chemists of other countries who were made honorary members are: H. W. Matheson, Canada; Georges Patart, France; S. P. L. Sorensen, Denmark; Heinrich Schicht, Czechoslovakia; Fritz Haber, Germany; Mario G. Levi, Italy; Toyokichi Takamatsu, Japan; A. M. Llopis, Spain, and G. Engi, Switzerland.

DR. ARISTIDES AGRAMONTE, of New Orleans, has been elected president of the fourth Pan-American Medical Congress to be held next year in New Orleans. Vice-presidents elected are: Dr. Hugh S. Cumming, Surgeon-General, Washington, D. C.; Dr. Charles Mayo, of Rochester, Minn.; Dr. J. G. W. Greef, of New York; Dr. John M. McReynolds, of Dallas; Dr. L. H. Bauer, of New York; Dr. Charles Snyder, of Miami; Dr. Rafael Silva, of Mexico City; Dr. Nicholas A. Solano, of Panama; Dr. Luis Bazetti, of Venezuela; Dr. Fernando Rensoli, of Cuba; Dr. Carlos Paz Soldan, of Peru; Dr. Victoriano Rodriguez Barahona, of Cuba; Dr. Ulysses Valdez, of Mexico City, and Dr. Horacio Ferrer, of Cuba.

DR. JAMES R. ANGELL, president of Yale University, will receive the degree of D.Litt. from the University of Liverpool in December. The degree of D.Eng. will be conferred on Dr. H. Hele-Shaw, emeritus professor of engineering at the University of Liverpool.

THE Remington Medal of the Philadelphia College of Pharmacy and Science has been awarded to Ernest F. Cook by the New York branch of the American Pharmaceutical Association. The medal is awarded annually to the person "who has done the most for American pharmacy during the preceding year, or during a longer period of outstanding activity and of fruitful achievement." The presentation will be made next fall.

DR. LOUIS J. RETTGER, dean of the science department of the Indiana State Teachers College, has been elected vice-president of the institution. Dr. Rettger has been a member of the faculty for forty years.

MR. JOHN G. BARRY, consulting mining geologist and engineer, has been appointed president of the Texas College of Mines and Metallurgy, El Paso.

MR. ASHER HOBSON, director of the foreign agricultural crop and market information service of the U. S. Department of Agriculture, has been appointed professor of agricultural economics at the University of Wisconsin. He will be in charge of cooperative marketing. Mr. Hobson was for seven years American representative at the International Institute of Agriculture in Rome.

DRS. CECIL ROBERT BROLYER and DANIEL KATZ have been promoted to be assistant professors of psychology at Princeton University. Dr. Henry Eyring, lecturer in chemistry at the University of California, and Dr. Wendell W. Mayer, international research fellow at the University of Munich, have been appointed research associates in chemistry.

THOSE promoted to the rank of professor at the

University of Missouri include Dr. Harry H. Charlton, in anatomy, and Dr. Adrian J. Durant, in veterinary science. The following appointments are also announced: Dr. Edgar D. Baskett, associate professor of psychiatry; Clarence Mitchell Tucker, of the Florida Experiment Station, associate professor of botany and plant pathologist.

DR. GEORGE HERMANN, of the School of Medicine of Tulane University, has accepted the professorship of clinical medicine at the University of Texas.

MR. HUMPHREY JOHN DENHAM, of Balliol College, has been appointed director of the agricultural engineering research institute of the University of Oxford.

DR. THEODORE H. FRISON has been appointed chief of the Illinois State Natural History Survey by the Illinois State Board of Natural Resources and Conservation. Dr. Frison had been acting chief of the survey since the death of Professor Stephen A. Forbes in March, 1930.

DR. H. W. VON LOESECKE, of the Bureau of Chemistry and Soils, has left Washington to take charge of the Citrus By-products Laboratory, now nearing completion at Winter Haven, Florida. Dr. W. W. Skinner, assistant chief of the chemical and technical unit, and Dr. F. C. Blanck, in charge of the food research division of the Bureau of Chemistry and Soils, will have general supervision. They will study methods of preserving orange and grapefruit by-products, and of utilizing orange and grapefruit wastes as stock and poultry feed.

DR. WALTER S. MOODY retired from active engineering work in the transformer departments of the General Electric Company on July 1, after forty-three years of continuous direction of the design, production and application of transformers. Mr. Moody was director of the Allegheny Steel Company and has for over thirty years directed the cooperative efforts of the General Electric Research Laboratories and the Allegheny Steel Company.

DR. LOUISE PEARCE, of the staff of the Rockefeller Institute for Medical Research, has been appointed visiting professor of syphilology at Peiping Union Medical College, Peiping, China, for six months, beginning on October 1.

DR. L. R. JONES, professor of plant pathology at the University of Wisconsin, sailed about August 1, to spend several months in the Orient. He plans to visit Japan, China, the Philippines and Hawaii.

DR. CONSTANCE E. HARTT, who has been assistant professor and co-chairman of the department of botany at Connecticut College during the past year, is now engaged in research on potassium deficiency in sugar cane at the Experiment Station of the Hawaiian Sugar Planters' Association, Honolulu. Dr. Hartt holds the Sarah Berliner Research Fellowship of the American Association of University Women.

DR. VACLAV VOJTECH, of Czechoslovakia, a member of the Byrd Antarctic Expedition, has arranged to accompany Dr. Aleš Hrdlička, of the U. S. National Museum, on a visit to Alaska. Dr. Vojtech will investigate the geographical and geological conditions of the peninsula. Dr. Hrdlička will continue his search for Indian and Eskimo antiquities.

MR. Y. L. KENG, who has been studying the grasses of China at the Grass Herbarium of the U. S. National Museum, under a fellowship from the Rockefeller Foundation, has gone to the New York Botanical Garden to consult the Chinese specimens there. He will also visit the Gray Herbarium of Harvard University, and the Arnold Arboretum. He expects to return to Washington about August 20.

MR. LINCOLN ELLSWORTH, representing the American Geographical Society, and Commander Edward H. Smith, of the U. S. Coast Guard, are accompanying Dr. Hugo Eckener on his Arctic tour in the *Graf Zeppelin,* which left Friedrichshafen on July 24.

IT is announced by the Oxford University Exploration Club that the Hudson Strait expedition, under the leadership of Mr. H. M. Clutterbuck, has left for Canada to explore the island of Akpatok and the surrounding regions. The party will consist of eleven men, including four biologists, a geologist, a surveyor, a physicist and a photographer. The undergraduate organizer and originator of the scheme is Mr. C. J. D'Aeth (Balliol).

AT the invitation of the Mexican Government the United States has named the following official delegates to represent it at the third Pan American Medical Association Congress, which meets in the City of Mexico from July 26 to 31, under the auspices of the Mexican Department of Health: Dr. J. C. Anderson, State Commissioner of Health, Austin, Texas; Passed Assistant Surgeon H. F. Smith, a commissioned medical officer of the Public Health Service, now on duty in the office of the American Consul General in Mexico City; Dr. Irving R. Roth, 45 East 82d Street, New York; Dr. John O. McReynolds, Dallas, Texas; Dr. William Davis Gill, San Antonio, Texas; Dr. W. E. Howard, Dallas, Texas.

THERE assembled on July 25 at Matameck on the north shore of the Gulf of St. Lawrence near Seven Islands a conference on wild life in various aspects. It has been organized by Mr. Copley Amory, of Bos-

ton and Washington, who has a wild life preserve in this locality, and he is bringing to it, largely at his own expense, a body of naturalists from all over the world. Sir George Perley has left for Matameck with a group of scientific men who are in the government's employ at Ottawa. The conference was tendered a luncheon on July 22 by the Province of Quebec when they were welcomed by Hector Laferte, Minister of Colonization, Game and Fisheries.

MR. HENRY O'MALLEY, U. S. Fish Commissioner, arrived at Juneau, Alaska, on July 3, on the fisheries patrolship *Penguin,* accompanied by members of the Senate Committee on Wild Life Conservation, Mr. Paul Reddington, chief of the United States Biological Survey; Mr. Carl Shoemaker, secretary of the Senate Committee on Wild Life Conservation, several Congressmen, and Attorney-General Charles Sisson. The object of their visit is to study game and wild life in the territory, with a view to its conservation. The party will proceed from Juneau to Pribolof Islands, where the seal herd will be inspected, thence back to Bristol Bay, Alaska's largest center for the sockeye salmon, where it will make a survey of the administrative methods of protecting the salmon and the canning industry. The committee also expects to visit Kodiak Island to secure first-hand information on the bear situation. Later it will go to the moose pastures on Kenai Peninsula, and then cruise up Cook Inlet.

SOME information is given in *Nature* in regard to the program of the International Illumination Congress, which is to be held in Great Britain from September 1 to 19. Provision is made for visits to London, Glasgow, Edinburgh, Sheffield, Buxton and Birmingham, following which the sessions of the International Commission on Illumination will be held at Cambridge. An item of outstanding interest in the London program is the proposed trip to the Port of London, returning to the Tower of London and Westminster by river, so that the illuminated buildings on the riverside may be seen. Throughout the visits to the cities named, the technical sessions, at which more than a hundred papers will be presented, will alternate with agreeable trips and social events. During the proceedings at Cambridge a lecture will be given by Sir Arthur Eddington. Membership of the congress is open to any one interested in illumination on payment of a registration fee of £2. Those desiring to take part in the congress should communicate with the honorary general secretary (Colonel C. H. S. Evans, 32 Victoria Street, London, S.W.1).

THE third International Congress of Radiology, under the presidency of Dr. Antoine Beclere, of Paris, opened at the Sorbonne on July 26. The sessions lasted throughout the week. Mme. Curie was honorary president of the congress. Twenty-seven countries were represented. Dr. Albert Soiland, of Los Angeles, was chairman of the American delegation, which numbered forty-five members. A radiological exhibition was held in connection with the congress. Dr. Soiland presented an invitation, asking the congress to hold its next meeting in the United States, but owing to its greater accessibility, Zurich was chosen as the place of meeting of the fourth congress, which will meet in 1934.

THE will of the late Dr. John Osborne Polak, professor of obstetrics and gynecology at the Long Island College Hospital, leaves $350,000 to the hospital, $100,000 for the erection of a science laboratory and $250,000 for a building for private patients for maternity and gynecological cases.

ACCORDING to the *Journal* of the American Medical Association a grant of $100,400 for a three-year period has been set aside by the Commonwealth Fund for a study of the function of the kidney, to be directed by Dr. Alfred N. Richards, professor of pharmacology in the University of Pennsylvania School of Medicine. Dr. Richards has devised methods by which the kidney of the frog can be examined microscopically while it is still functioning in the living body. The program of research includes the continuance and extension of these studies and the establishment of a clinical unit where experimental findings can be correlated with observation of patients suffering from kidney trouble.

DR. FREDERICK L. HOFFMAN, of Wellesley Hills, Mass., has presented to the California Institute of Technology his entire collection of books and data on aeronautics with particular reference to accidents and accident prevention. The collection includes many rare items. Last year Dr. Hoffman presented his entire collection of books and data on the North American Indians to Mrs. Joseph Lindon Smith, chairman of the Welfare Committee, Federation of Women's Clubs. During 1930 also Dr. Hoffman presented many thousands of volumes on foreign countries to the Baker Library, Harvard University.

APPARATUS for taking electric readings involving the electric current and potential as it passes through a given section of the earth's crust, for determining geological structure and as a means of locating ores, minerals, etc., has been invented by Oliver H. Gish, of the department of terrestrial magnetism of the Carnegie Institution, to whom has been granted an American patent. Rights in the patent have been assigned by the inventor to Carnegie Institution.

AN everblooming rose, developed by Henry F. Bosenberg, of New Brunswick, N. J., it was an-

nounced recently, has won the first plant patent granted by the United States Patent Office under the new law providing such protection. The patent was assigned to Louis C. Schubert, proprietor of the Somerset Rose Nursery, of New Brunswick, and was obtained by O. M. Kile, of Washington, acting as special plant agent. It covers "a climbing or trailing rose," the patentable feature of which is its ever-blooming character. The owner of the patent has the exclusive right to reproduce, use or sell his invention or discovery throughout the United States and its territories for seventeen years, or to license others to do so.

AT a recent meeting of the board of trustees of the Ohio State University, the following resolution was approved: "That the interest of the University in the *Ohio Journal of Science* be reaffirmed, and that a more definite plan of cooperation with the Ohio Academy of Science in the publication of the *Ohio Journal of Science* be established, $1,000 from the maintenance fund to be appropriated for the use of the *Ohio Journal of Science,* for the college year 1931–1932."

A FOREST research institute has been made a division of the New York State College of Forestry at Syracuse. The purpose in its establishment is to give the work in forest research now carried on by the college in different sections of the state a definite entity and more effective direction. Research in forestry in New York is authorized under the charter given the college by the legislature of the state. The board of trustees of the college during the past eighteen years has, in carrying out the obligations of the charter, set up special divisions of the college, such as the State Ranger School at Wanakena, the Roosevelt Wild Life Forest Experiment Station and other experiment stations.

AMERICA'S first autogiro has been donated to the Smithsonian Institution by Mr. Harold F. Pitcairn, president of the American Autogiro Company. Mr. James G. Ray, vice-president of the company, landed the machine on the lawn in front of the Arts and Industries Building on July 22, and the machine was accepted by Secretary Abbot in the presence of a group of distinguished guests. It will be exhibited in Power Hall next to an early Wright Biplane.

A COLLECTION of graphic charts and anatomical specimens belonging to the Museum of Hygiene of Dresden has been loaned by the museum to the Life Extension Institute and is on exhibition at the head office in New York. The Dresden museum is devoted to the instruction of the general public in the conservation of health. In 1911 the First International Hygiene Exhibition was held in Dresden, which proved to be so successful that its founders and sponsors cooperated in the establishment of the museum as a permanent institution. It is the gathering place of hundreds of international scientific associations. The health and hygiene sections of the League of Nations meet there regularly. The anatomical specimens included in the loan exhibit to the institute have been prepared by a special process which renders them translucent, making possible complete visualization of the underlying vascular and bony structures.

DR. C. F. KORSTIAN, director of Duke Forest at Duke University, Durham, North Carolina, has been notified by Sven Petrini, Secretary-General of the International Union of Forest Research Organizations, Experimentalfaltet, Sweden, of the election of Duke Forest as a member of the International Union of Forest Research Organizations. In addition to Duke Forest there are six other forestry institutions belonging to the Union in the United States—the Yale School of Forestry; Harvard Forest; School of Forestry and Conservation, University of Michigan; Forest Soil Laboratory, Cornell University; the Forestry Division of the University of California, and New York State College of Forestry, Syracuse, New York. The International Union of Forest Research Organizations originated in 1929 when the International Union of Forest Experiment Stations adopted a new constitution extending its field to include all forest research. At the end of 1930 it had fifty-four ordinary and three associate members in more than twenty nations. Germany has the largest membership embracing eight institutions, the British Empire has six members and other foreign countries from one to three.

THE *Experiment Station Record* states that a gift to the state of New Jersey for administration as a dairy research station has been made of 1,100 acres of crop and pasture land, 270 Guernsey and 55 Holstein cattle, elaborate buildings and equipment. The donor is James Turner, of Montclair, a stockbroker in New York City. Arrangements have been made whereby the equivalent of the taxes on the property will continue to be available to the townships of Wantage and Beemerville in which the property is situated. The estate consists of two farms, one of 700 acres on which $500,000 is said to have been spent for improvements and the other 3 miles distant and containing 400 acres. It is expected that the new station will begin operations immediately. Four projects are contemplated, dealing with the breeding of cattle for milk production, cattle feeding, control of diseases and economic management. The residence on one of the farms provides an elaborate administration headquarters and laboratories, and other equip-

ment will be added from time to time. In the words of *New Jersey Agriculture,* "thanks to Mr. Turner's vision and generosity, New Jersey will now have one of the largest and most complete dairy experiment stations in the world."

IT is reported in *Nature* that the Association of British Zoologists discussed at its general meeting the question of the payment of fees to zoologists for expert advice. It is well known, as Professor E. B. Poulton says in a letter on behalf of the council of the association, that a somewhat unscrupulous public takes for granted the good nature [and affluence!] of zoologists in requesting their professional help without offering payment in return. Whether it be a matter of the identification of a species or the delivering of a popular lecture, both demand the expenditure of time and energy, which the expert could have devoted profitably to his own purposes. The council's proposal is that, in the interests of their science, zoologists should demand fees for the work of identifying specimens and giving lectures. They say that such a demand would enhance the respect felt for the science, just as medical advice tends to be valued according to the size of the fee.

DISCUSSION

REDUCTION OF OXYLUCIFERIN BY ATOMIC HYDROGEN

SOME luminous animals, notably an ostracod crustacean, *Cypridina,* contain a substance, luciferin, which oxidizes to oxyluciferin in aqueous solution containing oxygen. In presence of a second substance, luciferase, luminescence appears. Luciferase acts both as a catalyst, accelerating the oxidation of luciferin, and also supplies molecules which may be excited to emit light by the energy of oxidizing luciferin. Oxyluciferin in water solution can be reduced to luciferin by various hydrogenation procedures. The mechanism has been fully discussed by Harvey.[1]

During attempts to excite luciferase to luminesce by the energy of recombination of hydrogen atoms we have observed that dry oxyluciferin can be reduced to luciferin. The apparatus was a modification of that used by Urey and Lavin.[2] Atomic hydrogen was produced in a high tension discharge tube at low pressure and drawn over the material exposed about 30 cm from the discharge tube. Dry oxyluciferin alone gives no luminescence when luciferase solution is added to it, but dry oxyluciferin first exposed to atomic hydrogen becomes reduced to luciferin, which can then be detected by luminescence on adding an aqueous solution of luciferase. No luminescence appears in the atomic hydrogen treated oxyluciferin on adding water alone but only if luciferase is present also, as is to be expected. This experiment confirms the results obtained by reducing oxyluciferin in water solution and makes it quite certain that the luciferin-oxyluciferin change is a dehydrogenation-hydrogenation reaction.

When dry luciferase and luciferin are exposed to low concentration of atomic hydrogen there may at times be observed a faint bluish glow which breaks into incandescence on raising the atomic hydrogen concentration. Dry egg albumen and dry powdered pill-bugs (*Oniscus*) exhibit a dull orange glow in low concentration of atomic hydrogen which may also pass into undoubted incandescence. The faint glow of *Cypridina* may be a luminescence or a low temperature incandescence since the containing vessel does become warm. Willemite and certain other substances show undoubted luminescence in atomic hydrogen.

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1 *Bull. Nat. Res. Council,* No. 59, p. 50, 1927.
2 *J. Amer. Chem. Soc., 51,* 3286, 1926.

"THE POSSIBLE RÔLE OF MICRO-ORGANISMS IN THE PRECIPITATION OF CALCIUM CARBONATE IN TROPICAL SEAS"

THE statement made by Dr. Werner Bavendamm, in the issue of SCIENCE for May 29, 1931, on the subject quoted above, compels me to make some critical comments. Much as I detest polemics I feel it necessary that those interested in the subject in question have the facts before them.

Dr. Bavendamm discusses at some length the observations in tropical seas with respect to calcium carbonate precipitation, and makes it appear that his findings are in conflict with mine. As a matter of fact, Dr. Bavendamm confirms practically all the results obtained in my own studies as reported elsewhere.[1] For example, (1) Dr. Bavendamm finds a very small bacterial population in the open sea. This confirms my findings and those of others. (2) Dr. Bavendamm shows that the bacterial population of muds like those off the Bahama Banks is relatively small as compared with those of soil populations. This also confirms my findings. (3) Dr. Bavendamm

1 Publication No. 340, Carnegie Institution of Washington; Publication No. 391, Carnegie Institution of Washington, 1929.

states that investigators have used erroneously the term "calcium bacteria." This is a point which, among several, I emphasized particularly in my papers when I stated that the literature of bacteria shows that many different forms of bacteria have the power of precipitating calcium carbonate under the proper conditions.

In view of these statements by Dr. Bavendamm, I fail to see why he does not agree with what he calls my ideas and conclusions "based on experiments which were not sufficiently convincing." What Dr. Bavendamm probably means is that my experiments are not sufficiently convincing to him.

It appears as I read Dr. Bavendamm's statement that he bases all his views and attitude on the problem in question on his findings of bacteriological conditions existing in mangrove swamps like those on the coast of Williams Island. The fact that bacteria and other micro-organisms exist in large numbers and in great variety in mangrove swamps is not in the least surprising to me, nor can it be to any one who is acquainted with bacterial populations in such material as exists in mangrove swamps. To be sure, very little investigation of bacterial populations in such mixtures of organic and inorganic materials has been carried out, and it is highly desirable that much of this work shall be done, but this has little or no relation to the question as to whether or not the calcareous deposits of the earth's surface have been built up through bacterial action in the open sea.

If Dr. Bavendamm will consult my papers he will see that I have merely examined critically the possibility of calcuim precipitation in any quantity in the open sea through bacteria existing there, and with special reference to the Drew hypothesis. I have never claimed that calcium carbonate precipitation could not be effected in such a medium as the mangrove swamps, nor, if Dr. Bavendamm reads my papers carefully, will he find the conclusion that the physical-chemical method of calcium carbonate precipitation to which he referred was regarded by me as the only method of calcium carbonate precipitation in the sea. My main contention was, and still is, that no case has as yet been made out for specific forms of bacteria which have as their function the precipitation of calcium carbonate, and secondly, that no case has ever been made out for large-scale precipitation of calcium carbonate in the open sea, by the mechanism postulated by Drew and too hastily approved by geologists, generally speaking.

That many living organisms are concerned with the secretion of calcium carbonate has been emphasized by many biologists. That these may have been indirectly concerned with the accumulation of calcium carbonate deposits has also been emphasized by many investigators, but these facts have no bearing on the points originally made by Drew with which I took issue in my publications in this field.

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INHERITED TASTE DEFICIENCY

IN SCIENCE for April 17, 1931, Dr. Arthur L. Fox, of the laboratories of the du Pont de Nemours Company, was reported as having found that certain persons apparently have no ability to taste para-ethoxy-phenyl-thio-urea. It was reported that 40 per cent. of the individuals tested could not taste the compound, while to the remaining 60 per cent. it was exceedingly bitter. I immediately wrote Dr. Fox asking for some of the compound with which to investigate the possible inheritance of this taste deficiency. This is a preliminary report of the occurrence of the condition in one hundred families.

First of all, I can confirm Dr. Fox's conclusion that the taste deficiency actually exists, and is not a matter of age, sex nor race. It is not dependent upon acidity nor alkalinity of the mouth. Those tasting it find it bitter, usually exceedingly bitter, even nauseating, while those not tasting it are unable to get any taste at all, even after rinsing the mouth with dilute acids or alkalis.

My results to date show 68.5 per cent. tasters, and 31.5 per cent. with the taste deficiency. I have tried it out in families, and the results of the first one hundred families are so conclusive that they are worthy of record. The taste deficiency is apparently due to a single recessive gene. It is not sex-linked nor sex-influenced. When neither parent can taste the compound, none of the children can taste it.

Dr. Fox tells me that the taste deficiency occurs in other compounds of the phenyl-thio-urea group as well. Di O-tolyl-thio-urea behaves somewhat differently from the others, and will be reported on later. For the present it is sufficient to establish the taste deficiency as a unit-factor recessive.

The results of the study of one hundred families are as follows:

		Children	
	No. of families	Can taste	Can not taste
Both parents can taste	40	90	16
One parent can taste, the other can not	51	80	37
Neither parent can taste	9	0	17

	Males	Females	Total	Percentage
Can taste	150	151	301	68.5
Can not taste	71	68	139	31.5

Further work is being done on the inheritance of this taste deficiency, its linkage relations and its physiology, and these results will be reported in detail in the near future.

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GETTING THE STUDENT TO USE HIS OWN INTELLECT

PROFESSOR WILLIAM A. RILEY in his timely address as chairman of the Section of Zoology of the American Association for the Advancement of Science reverts to the advice given by Huxley in 1869 that, "if the great benefits of scientific training are sought, it is essential that such training should be real; that is to say, that the mind of the scholar should be brought into direct relation with the fact, that he should not merely be told a thing but made to see by the use of his own intellect and ability that the thing is so, and not otherwise."

In discussing the relation of this advice to the present educational situation Professor Riley incidentally raises a question without answering it. The question is how far laboratory work as now carried on in secondary schools and colleges is essential to the purpose of inducing students to use their own intellects. This topic has recently aroused a good deal of interest among teachers of science and is one which deserves serious consideration.

The usual way of conducting laboratory work is to put into the hands of the student a laboratory manual or sheet of directions which he must follow in order to produce the expected result. Subsequently he reads an assignment in a text-book and listens to a lecture. If this is the best possible way to get the student to do his own thinking it fully justifies the expenditure of money, space and time needed for the equipment of the laboratory and the conduct of laboratory courses. If it is not the best way and classroom demonstrations, lectures and text-books will serve equally well, then the expenditure is not justified.

Possibly the question might be answered conclusively if the same kind of subject-matter were presented to two classes equal in numbers, age and intellect but working according to the two different methods and at the end of a definite period tested with regard to their ability to solve problems involving application of what they had learned. Failing such evidence there may yet be some profit in discussing the probable relative advantages and disadvantages of the two methods in the light of experience both educational and practical.

In actual life more than one kind of thinking is needed. The successful chauffeur or cook must be able to see relations between concrete, material things and swiftly draw the correct conclusions. To me the probabilities are in favor of this ability being developed and strengthened more effectively if the chauffeur or cook actually drives a car or prepares a meal than if he simply watches a demonstration or hears an explanation. A combination of the two methods would be still more effective. Similarly if, in teaching science, we as educators wish to develop and strengthen the habit of thinking with the aim of producing particular effects on concrete material, a combination of individual laboratory experience with demonstration and explanation is advantageous. If children are being taught botany with the object of becoming better gardeners or farmers there is a real advantage in individual observation and experimentation with seeds and seedlings over demonstration with charts and models. A future practical chemist will benefit more by handling apparatus and reagents than by watching some one else do the same thing.

When the educational problem is less definite, when science is being taught not with the immediate aim of training for a specific occupation but of developing general mental powers, of establishing a scientific attitude, of giving practice in dealing with abstract as well as concrete ideas and of enabling the student to understand everyday happenings, what are the advantages, if any, of individual laboratory work? Will, for example, powers of observation and comparison be developed better if each student is supplied with specimens of plants or animals and asked to make a list of similarities and differences or if the teacher points them out on a chart or model? Which will leave a more lasting impression of the fact that heat causes gases to expand, for the children to make bubbles rise through water themselves or to watch the teacher do it?

The answer seems obvious. The secret of the greater effectiveness of individual laboratory work lies in the increased motivation. Most young people love to be active and to bring about results by their own efforts. Interest is greatly enhanced if the problem is sufficiently simple for them to devise their own method of solving it. This has the additional advantage of helping to establish the habit of self-reliance.

I believe it is a fact that some laboratory courses do not stimulate initiative and interest and I believe that this is because the laboratory directions make no appeal to the student. They are carried out simply as a matter of routine in order to prepare for the lecture or quiz which is to follow. In other words, the chief consideration has been the logical development of subject-matter rather than the stimulation of the student's initiative and powers. The reverse

process is better suited to encourage independent thinking. When the student's interest and initiative have been aroused he can the more readily be led to realize the necessity of logical arrangement of ideas.

Since pupils differ widely in ability to absorb and to utilize knowledge and also in the kind of lives they will lead after leaving school and college, the real success and value of any method will depend on its adaptation to the personality of the pupil. The ancient method of professorial lecturing and student note-taking is noticeably lacking in objectivity and individualization. The opposite extreme of detailed and minute laboratory study of specimens and processes achieves great objectivity at the expense of perspective and broad understanding. It fosters habits of mental short-sightedness. A middle course is indicated, individual laboratory work suited to the student, supplemented by reading, demonstrations and discussion in which the student has an opportunity to take part.

The question has its economical aspect also. When a limited sum of money is apportioned to departments of science an obligation exists to expend it advantageously. Duplication of simple, inexpensive apparatus easily manipulated by the students and the purchase of single pieces of more complex apparatus for purposes of demonstration would seem best.

The entire abandonment of individual laboratory work would surely be antagonistic to the purpose of mental development. Research is unnecessary to prove that point. Investigation to determine beneficial modifications of present laboratory practice in order that it may be better adapted to the interests and needs of the students would be more to the point.

M. LOUISE NICHOLS

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POSITIVE GAS PRESSURE IN POPLAR

IN line with a recent article in SCIENCE entitled, "Positive Gas and Water Pressure in Oaks," by C. A. Abell and C. R. Hursh (SCIENCE, 1895, p. 449), I am reminded of three cases of positive gas pressure, all in large trees of *Populus tacamahaca* Miller (*P. balsamifera* L.) in a recent summer in northern Michigan. In all three cases there was a distinct hiss as soon as the instrument borer went in about 2–3 cm, which continued during most of the rest of the boring. The pressure was not sufficient to force the core out of the increment borer and could be heard only in the vicinity of the tree. One of these trees, which was 40.6 cm in diameter, was cut down. This tree was sound throughout and bled very actively from the stump.

Hundreds of borings on the two aspens (*Populus tremuloides* Michx. and *Populus grandidentata* Michx.) in no case were accompanied by any evidence of positive pressure.

FRANK C. GATES

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SOCIETIES AND ACADEMIES

THE NORTH CAROLINA ACADEMY OF SCIENCE

THE thirtieth annual meeting of the North Carolina Academy of Science was held at State College, Raleigh, N. C., on May 8 and 9. Papers were presented before the general section of the academy on Friday morning and afternoon. On Friday evening the retiring president, W. F. Prouty, professor of geology in the University of North Carolina, gave the presidential address on "The Origin of Folded Mountains." On Saturday morning the academy met in the following sections: general section, chemistry section, mathematics section and physics section. Eighty papers and twenty-four exhibits were on the program. (Abstracts of most of these and complete papers of several will appear in an early number of the *Journal of the Elisha Mitchell Scientific Society*.)

Resolutions of respect were passed in honor of two deceased members, William Cain, Kenan professor emeritus of mathematics in the University of North Carolina, and John William Nowell, professor of chemistry in Wake Forest College.

The executive committee reported the election of thirty-four new members during the year, and the reinstatement of eight former members. One hundred and eighty-six registered at the meeting.

Walter Burke Davis, a student of the Greensboro Senior High School, was declared the winner of the high-school science prize, a silver loving-cup, for the best essay submitted by a high-school student. (Essays for 1931 were confined to the fields of biology and geography.)

The officers elected for the year 1931–32 were:

GENERAL ACADEMY

President, F. A. Wolf, Duke University.

Vice-president, W. E. Speas, Wake Forest College.

Secretary-treasurer, H. R. Totten, University of North Carolina.

Executive Committee, the above officers; Bert Cunningham, Duke University; W. L. Porter, Davidson College; F. W. Sherwood, N. C. Agricultural Experiment Station.

Representative to the A. A. A. S., H. R. Totten, University of North Carolina.

CHEMISTRY SECTION

Chairman, L. A. Bigelow, Duke University.
Vice-chairman, F. W. Sherwood, State College.
Secretary-treasurer, H. D. Crockford, University of North Carolina.
Councilor, L. G. Willis, State College.
Executive Committee, A. S. Wheeler, R. W. Bost and W. C. Vosburg..

MATHEMATICS SECTION

Chairman, E. T. Browne, University of North Carolina.
Secretary, E. R. C. Miles, Duke University.

PHYSICS SECTION

Chairman, J. L. Lake, Wake Forest College.
Secretary, Calvin Warfield, North Carolina College for Women.

The thirty-first annual meeting of the North Carolina Academy of Science will be held at Wake Forest College, Wake Forest, N. C., in the spring of 1932.

H. R. TOTTEN,
Secretary

THE VIRGINIA ACADEMY OF SCIENCE

THE Virginia Academy of Science held its ninth annual meeting in Norfolk, on April 24 and 25 with a registration of 242.

The evening address to which the public is particularly invited was delivered by Dr. William A. Kepner, of the University of Virginia, on the subject "A Modern Drift in Biological Thought." There were 111 papers read before the various sections.

A new section was authorized at this meeting—a section on medical sciences. It is expected that the new section will function along the lines of Section N of the American Association for the Advancement of Science. Physicians in Virginia who are interested in the fundamental medical sciences, such as anatomy, bacteriology, biochemistry, embryology, pathology, pharmacology and physiology, will be welcomed to membership in the academy and to participation in the activities of this and other sections.

It is confidently expected that this new section will grow rapidly, as it offers scientifically inclined physicians an opportunity to read scientific papers before an appreciative audience, to take part in stimulating discussions and to become acquainted with other like-minded scientific men.

Dr. I. D. Wilson, of the Virginia Polytechnic Institute, was elected president for the coming year, and Dr. H. E. Jordan, of the University of Virginia, new member of the council.

E. C. L. MILLER,
Secretary-Treasurer

SCIENTIFIC APPARATUS AND LABORATORY METHODS

RUTHENIUM TETROXIDE AS A FIXATIVE IN CYTOLOGY

IN the preparation of certain tissues for microscopic examination, use is frequently made of osmium tetroxide as a fixing or killing agent. This use is largely to avoid coagulants which would materially change the natural structure of the protein constituents of the cell. This note is to call attention to the possibility of the use of ruthenium tetroxide for this purpose, inasmuch as this compound is one of the two examples of the highest state of oxidation known, the other being the corresponding osmium compound.

Ruthenium tetroxide decomposes very readily and is a very energetic oxidizing agent. It is difficult to prevent its decomposition in solution even when kept in the cold and in the dark. We have been most successful in this respect when saturated chlorine water has been used as solvent. Ruthenium tetroxide is supplied in sealed glass ampoules which may be crushed under the cold solvent with no difficulty or danger. It forms a golden yellow solution from which after several weeks a black deposit of the lower oxides of ruthenium separates, at which time its fixing properties have largely disappeared. A stock solution was prepared by breaking a one gram ampoule of the tetroxide under 100 cc of chlorine water. The tetroxide is not very soluble and the greater part remains undissolved so that a saturated solution with respect to the tetroxide is still maintained even after the majority has passed into the lower oxides. For use as a fixative, the stock solution was diluted about twenty times with either distilled water or a ½ to 1 per cent. formic or acetic acid solution.

The ruthenium tetroxide fixative was used extensively on pollen mother cells of *Tradescantia zebrina* (Hort) and closely compared with osmium tetroxide. The ruthenium salt was found to be extremely useful in obtaining the chromonematic structure of the chromosomes at all stages. The morphological results of these studies will be published elsewhere.[1] From our experiences it appears that ruthenium tetroxide is preferable to osmium tetroxide when used for the purpose described. The advantages are, however, partly offset by the fact that ruthenium tetroxide will

[1] B. R. Nebel, "On the Structure of the Chromosomes in *Tradescantia zebrina* (Hort.)," *Zeitschr. f. Zellforschg.* In press.

not penetrate deeply into several layers of tissue, as is possible with the osmium salt. It fixes the outer cell layers very well but it appears to have been mostly decomposed before cells in the interior of a structure can become fixed. Methods to overcome this difficulty are being studied.

To familiarize the reader with the use of the fixative, the following general procedure is given—(1) Smear anthers between two slides, (2) immediately drop diluted fixative onto slides and leave for 3 minutes, (3) pour off fixative and replace with one drop of Linder's medium (glycerine 40, lactic acid 20, phenol 20 and water 20 per cent.), (4) cover and seal. The fixative should render the material distinctly gray (but not black) during the fixation process when viewed against a white background. Under the microscope the chromonemata appear dark against the gray protoplasm. If staining is desired, a small amount of carmine may be added to Linder's medium or the slides may be dehydrated after fixation and stained by other methods. Treatment with H_2O_2 was found to be distinctly detrimental to maintaining the fixed cell structures.

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A METHOD FOR LOCATING THE LARVAE OF THE MOSQUITO MANSONIA

ENTOMOLOGISTS and sanitary engineers engaged in mosquito work often experience difficulty in locating the breeding places of *Mansonia perturbans* Walk. even though the abundance of this mosquito at the particular time and place is such as to make it a very serious pest. The writer has been engaged in investigations of the biology of *Mansonia* in central Florida for the past two years and has developed the method here described for collecting larvae of this insect. Of several methods tried, this has proven the most satisfactory in locating breeding grounds of *Mansonia*.

The larvae of *Mansonia* differ from those of other mosquitoes in that, with the exception of the first few days of larval life, the larval and pupal periods are spent at the bottom of the ponds and marshes where they breed. Peculiar adaptations of the larval air tube and of the pupal breathing trumpets enable them to pierce submerged roots and stems of plants and obtain air therefrom. Difficulty in locating the breeding grounds has undoubtedly arisen on account of the fact that the larvae quickly detach from stems and roots when disturbed and bury themselves in the débris at the bottom of the pond. Thus very rarely are they found by merely examining submerged stems and roots which have been pulled out of the water.

Actually to determine whether or not a marsh is breeding *Mansonia*, the plants over a small area (in practice about one square yard) should first be pulled up, thus disengaging any larvae that may be attached thereto; immediately after which the débris from the bottom of this area, in which if present the larvae are hidden, should be scooped out to a depth of about one inch. This may be done by means of a vessel having a screened bottom. A regular water bucket, the bottom replaced by twenty mesh screen wire, has proven satisfactory for this operation. As each scoop of débris is collected it is placed in a twenty mesh screen wire basket which is held partly submerged and holds in captivity any larvae thus collected. By keeping this basket partly submerged and by occasional shaking a large quantity of mud and minute trash is washed out, thereby lessening the quantity of débris to be examined later. This wire basket may be of any shape, but one recommended on account of ease of construction is conical, having a diameter at the mouth of eighteen inches and a depth of twenty-four inches.

The basket with its contents is next carried ashore for examination. The procedure usually followed in examining the débris is to place a small handful of it in a white enameled laboratory pan, adding about one quart of clear water, and then carefully to search the pan for larvae. If present, the larvae, which are whitish and very active, will be found at the bottom of the pan. Often, however, some individuals, usually those which have been injured by rough handling, are found at the surface of the water.

The number of these examinations necessary to determine whether or not a given area is infested with *Mansonia* will, of course, depend on the size of the marsh and on the number of different types of environment present therein.

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SPECIAL ARTICLES

ON ATMOSPHERIC ELECTRICITY

ACCOUNTS of certain remarkable effects of atmospheric electricity on the growth of plants have been related by enthusiasts both in this country and abroad. Yield increases of more than thirty per cent. have been reported from fairly definite and systematic yield tests. The results of other investigations have been negative, no significant yield increases having

been obtained through the use of the electrical charge as secured from the air.

In order to check some of these results, experiments were instituted at the Agronomy Farm of Iowa State College by the departments of farm crops and soils and of physics, during the spring and summer of 1930.

No significant differences in yields were obtained as a result of the first year's trials. However, these tests were not taken as very conclusive as the summer was very dry and the amounts of charge obtained throughout the period were exceedingly small. The tests are to be continued for the coming season.

During the course of the experiments some effects of a purely electrical nature appeared which it seemed worth while to investigate further. It has been found by several investigators that there is a continual current of electricity flowing between the earth and the air. The work of C. T. R. Wilson (*Phil. Trans.*, 221: 73, 1921) (*Proc.* Roy. Soc., A vol. 80, p. 539, 1908), Schonland (*Proc.* Roy. Soc., A vol. 118, p. 229, 1927), Swan, (*I. C. T.* VI, 442), and others indicate that the direction of the positive current is from the air to the earth during fair weather, *i.e.*, the earth is losing negative charge, and that during storm periods, the current is more often in the other direction. According to Wilson (*Proc.* Roy Soc., A vol. 80, p. 539, 1908), and Gerdien (*Physikal. Zeit.*, p. 647, Jahr. 6, 1905), this fair-weather current for the whole earth amounts to about 1,000 amperes.

The plot of ground under investigation in our experiments was one hundred and fifty feet square. This was wired about twelve inches under ground with copper radio cable, the wires extending entirely across the plot north and south, being spaced thirty feet apart east and west. The apparatus used for collecting the charge from the atmosphere consisted of one original Christofleau apparatus (*Bull.* of the Etablissement J. Christofleau Industriel) and four variations, three of the Vincent type and one of our own. These were essentially brushes of wires on an iron standard, the brushes containing from eighteen to twenty-seven wires. The lengths of the wires varied from approximately six inches to eighteen inches and they were so arranged on the standard that the tops of the wires were about even. The diameters of the brushes varied from about eight inches to fourteen inches. Our design was slightly different, consisting of six brass bars eighteen inches long, radiating from a central plate and carrying several copper wires projecting upward. These "heads" were mounted on 4″ x 4″ poles twenty feet high, bolted permanently to posts set solidly in the ground. The heads were insulated from the poles by a resistance composition of slag oil and clay made by the Vulrox Co., of St. Louis. The connecting wires leading down the poles were mounted on glazed insulators. These were connected through a series of switches to one plate of a high grade mica condenser, the other plate being connected to the ground wires. A high sensitivity Leeds and Northrup ballistic galvanometer was used to measure the charges collected. Another Leeds and Northrup R type high sensitivity galvanometer was installed to measure the current directly, but it was useless during the summer except during showers when the currents became sufficiently large to produce appreciable deflections.

The poles carrying the collector heads were erected along the south side of the plot under investigation, and about two feet inside a woven wire fence which inclosed the south and west sides of the plot. There were several tall trees to the north of the plot but as none of these were within 160 feet of our apparatus, it was not thought that they would materially affect the results. A cinder road ran along the south side of the plot and on account of the very dry weather of the last summer, was usually quite dusty. Clover and small grain were grown across the road and corn to the east of the plot. The plot itself contained corn, soy beans, turnips, garden beans, and swiss chard. The measuring instruments were housed in a sheet-iron shack at the southwest corner of the plot.

From June 25 to July 10 the currents were upward, *i.e.*, the ground was gaining negative charge. These currents were small, being of the order of 5×10^{-9} ampere. This is about one hundred times the average fair-weather current as given by Wilson but as points were used here as dischargers, larger results were to be expected. The last rain of the season of any considerable quantity occurred on July 4 so that by July 10 the ground was quite dry and remained so until about the middle of September. The summer was very hot, the temperature on several days reaching 98° F. or higher during the afternoon. During the interval from July 11 to September 6, when readings at this location were discontinued, the prevailing direction of the currents (except during light showers) was downward. The currents during this period were very small being of the order of 5×10^{-10} ampere.

Late in October, the apparatus was dismantled and three of the heads mounted on the top of the physics buildings. The height of the heads above the ground was about thirty feet. The mounting was similar to that used previously except bakelite replaced the slag oil composition as insulator and a ground plate replaced the long ground wires. Several small rains had fallen in the interval between the last readings taken at the farm and the installation of the instru-

ments on the building. The currents during most of the time since have been large enough to measure directly with the type R galvanometer, or of the order of 5 x 10^{-9} ampere. These are about the same as those of June, 1930, and in the same direction, *i.e.*, upward currents.

It will be noted in this connection that our results, except during the very driest part of the last summer, are in direct contradiction to the fair weather results of the investigators mentioned above. Our results indicate that in this vicinity the earth is nearly always gaining negative charge. Our results check the others for the prevailing direction of the current during a rain, *i.e.*, that the earth is gaining negative charge. It was also noted that, during a thunderstorm, the direction of change of field during a lightning stroke, was predominately in one direction.

Most of the changes noted occurred when the storm was overhead so that the distance to the points of discharge were comparatively short. All the changes in field—with two exceptions—were positive, *i.e.*, the fields set up by the discharges were downward. One of these two exceptions occurred as the storm was approaching and was quite small. The other occurred while the storm cloud was overhead and was comparatively strong. These results check those of Schonland and Craig for near discharges. (*Proc.* Roy. Soc., A vol. 114, p. 229, 1927). As there was only one observer and he was continually occupied with the measuring instruments and as the storm was approaching from the opposite side of the building, there was no opportunity to note the character of the discharges or to make any actual estimates of the distances to the points of discharge.

Thinking that there might possibly be some error in our method of determining the direction of the current, an electrometer was set up and the direction again determined. Both galvanometers and the electrometer gave the same results. Frequent tests were also made to determine whether the direction of the currents noted might not be due to small e.m.f.'s developed in the switches but no evidence to this effect was found.

While the apparatus was set up in the field, it was noted that whenever an auto passed stirring up a cloud of dust which drifted across the apparatus, there was an added current set up. This was always in one direction and was interpreted as being due to tribo-electricity generated between the rubber tires of the car and the cinders on the road. Without exception, however, this was followed at an interval of about thirty seconds by a reverse current. This reverse current has not been explained.

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USE OF PROTOZOA IN MEASURING THE NEUTRALIZING VALUE OF COBRA ANTI-SERUM[1]

IN previous studies of the effect of toxins and venoms on protozoa[2] it has been shown that paramecia may be used in measuring the potency of venoms. Attempts were made to use paramecia in measuring the neutralizing value of the anti-serum of Crotalus (rattlesnake) venom, but results were not wholly satisfactory. More recently the study has been extended to the use of paramecia in measuring the strength of cobra anti-serum. In this the results have been more satisfactory. It is the purpose of this paper to describe the methods used and the results obtained in ten different titrations where paramecia were used in measuring the neutralizing value of a sample of cobra anti-serum.

The method which was used in measuring the strength of the cobra anti-serum consisted in placing paramecia (in each case four animals in 1 cc) in various mixtures of cobra venom and anti-serum and thus determining the least amount of anti-serum required to protect the animals from a given amount (0.000005 gram) of venom. The method and results of ten different titrations are shown in the accompanying table.

The venom which was used in these titrations was supplied in desiccated form by the Pasteur Institute. A stock solution of this venom was made by dissolving 0.05 gram of the dry venom in 9 cc of distilled water to which 1 cc of glycerine had been added. This solution was brought to a pH of 7.0 by the addition of Na_2HPO_4. This stock solution did not deteriorate in strength during the period of the investigation and was used in making all dilutions of venom indicated in the accompanying table. The solution was kept at from 5 to 10° C. Repeated titrations showed that the least concentration (minimal lethal concentration) of this venom required to kill paramecia (*P. multinucleatum*) was 0.000002 gram per cc and that the maximum tolerance of this species of paramecia for the venom was 0.0000016 gram per cc of medium.

The anti-serum used in these titrations was a sample supplied in desiccated form by the Pasteur Institute. A fresh stock solution of the desiccated anti-serum was made up for each titration shown in the accompanying table. This, in each case, was made by weighing 0.05 gram of the desiccated anti-serum (the equivalent in this case of 0.5 cc of liquid anti-serum) and dissolving in 10 cc of neutral distilled water. From such stock solution all dilutions of the anti-serum shown in the table were made.

[1] This study was aided by a grant from the National Research Council.

[2] C. H. Philpott, *Jour. Exp. Zool.*, vol. 56, No. 2; *Jour. Morph. and Physiol.*, vol. 46, No. 1; *Proc.* Soc. Exp. Biol. and Med., vol. 26; *Biol. Bull.*, vol. 60, No. 1.

TEN TITRATIONS OF COBRA ANTI-SERUM IN WHICH PARAMECIA WERE USED AS TEST AGENTS

Amount of cobra venom per cc of mixture	Amount of serum per cc of mixture	Number of paramecia added to 1 cc of mixture	Number of animals remaining alive after 1 hour. Temperature 20° C.									
	Anti-serum		Titration number									
gram	cc		1	2	3	4	5	6	7	8	9	10
0.000005	0.0005	4	0	0	0	0	0	0	0	0	0	0
"	0.0010	4	0	0	0	0	0	0	0	0	0	0
"	0.0014	4	0	0	0	0	0	0	0	0	0	0
"	0.0019	4	2	0	3	0	1	0	0	3*	0	1*
"	0.0024	4	4	0	3	0	2	2	0	3	1	1
"	0.0029	4	4	4	4	3	4	4	0	4	4	4
"	0.0033	4	4	4	4	4	4	4	4	4	4	4
"	0.0038	4	4	4	4	4	4	4	4	4	4	4
	Normal horse serum, cc											
"	0.0005	4	0	0	0	0	0	0	0	0	0	0
"	0.0010	4	0	0	0	0	0	0	0	0	0	0
"	0.0014	4	0	0	0	0	0	0	0	0	0	0
"	0.0019	4	0	0	0	0	0	0	0	0	0	0
"	0.0024	4	0	0	0	0	0	0	0	0	0	0
"	0.0029	4	0	0	0	0	0	0	0	0	0	0
"	0.0033	4	0	0	0	0	0	0	0	0	0	0
"	0.0038	4	0	0	0	0	0	0	0	0	0	0

* Animals in pathological condition.

The medium used in making all dilutions from the venom and anti-serum stock solutions consisted of a 0.025 per cent. solution of beef extract in non-toxic distilled water. This medium was brought to a pH of 7.3 by the addition of Na_2HPO_4 and was kept in sterile condition until time for use.

The mixtures of venom and anti-serum which are described in the accompanying table were allowed to stand at 5° C. for from 10 to 60 minutes before testing their effects on paramecia. It was found that under these conditions neutralization of the venom was as complete in 10 as in 60 minutes. It was also found that the venom in these mixtures deteriorated to some extent when the solutions stood at room temperature and in the presence of sunlight.

The animals used in these titrations came from a culture of *Paramecium multinucleatum* obtained from Dr. L. L. Woodruff, of Yale University. All animals used were the descendants of one animal isolated at the beginning of the investigation.

It is evident from an inspection of the data in the accompanying table that the least amount of the anti-serum required to sufficiently neutralize 0.000005 gram of venom so that paramecia are able to live in the mixture is from 0.0019 to 0.0029 cc of the anti-serum per cc of mixture. Considering the fact that these animals are normally able to tolerate 0.0000016 gram of cobra venom per cc of mixture it follows that 0.0000034 gram of the venom is neutralized by from 0.0019 to 0.0029 cc of the anti-serum. From this it may be calculated that 1 cc of the anti-serum neutralizes from 0.0011 to 0.0017 gram of cobra venom. The Pasteur Institute, using warm-blooded animals as test agents, reported this anti-serum to have a neutralizing value of from 0.0009 to 0.00125 gram of cobra venom per cc. The titre obtained with the use of paramecia and that obtained by the Pasteur Institute, where warm-blooded animals were used in the test, are essentially in agreement.

It is the belief of the writer that the above described method of determining the strength of cobra anti-serum is reliable and that it could be used routinely.

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